THE PREVALENCE OF DIABETES MELLITUS AND PANCREATITIS COMPLICATIONS IN HIV INFECTED INDIVIDUALS IN NYERI DISTRICT

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR AWARD FOR DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY.
DECLARATION

I declare that the work presented in this thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This work is dedicated to my lovely wife Alice Watare Maina and to our wonderful kids Gamaliel Mugi Maina and Abigail Wacuka Maina.
ACKNOWLEDGEMENT

I appreciate the support of my supervisors Dr. Joseph N. Makumi, and Dr. J.J. Ngeranwa who assisted in the formulation, design and execution of this study. I also greatly appreciate the expert advice and guidance of Dr. Kimani MOH-Kerugoya and Wilfred K. Gatua, and Andrew Mboche from Laboratory Department, Kenyatta National Hospital and Laboratory Department, PGH Nyeri respectively.

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To God who enabled me complete this and indeed contribute something to science, may His name be praised.
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# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>μMol/l</td>
<td>Micromole per liter</td>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Amino Transferase</td>
</tr>
<tr>
<td>AMFAR</td>
<td>American Foundation of AIDS Research</td>
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<tr>
<td>B.M.I.</td>
<td>Body Mass Index</td>
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<td>C.C.C</td>
<td>Comprehensive Care Centre</td>
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<tr>
<td>D.M.</td>
<td>Diabetes Mellitus</td>
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<tr>
<td>E.D.T.A.</td>
<td>Ethylene Diamine Tetra Acetic Acid</td>
</tr>
<tr>
<td>E.M.C.V.</td>
<td>Encephalomyocarditis Virus</td>
</tr>
<tr>
<td>H.A.A.R.T.</td>
<td>Highly Active Anti-Retroviral</td>
</tr>
<tr>
<td>H.C.V.</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>H.I.V.</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>I.D.D.M.</td>
<td>Insulin Dependent Diabetes Mellitus</td>
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<tr>
<td>N.I.D.D.M.</td>
<td>Non Insulin Dependent Diabetes Mellitus</td>
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<tr>
<td>N.I.M.H.</td>
<td>National Institute of Mental Health</td>
</tr>
<tr>
<td>NAPAT</td>
<td>National Association of Pathologist Trust</td>
</tr>
<tr>
<td>P.G.H.</td>
<td>Provincial General Hospital</td>
</tr>
<tr>
<td>P.I.</td>
<td>Protease Inhibitor</td>
</tr>
<tr>
<td>R.T.I.</td>
<td>Research Triangle Institute</td>
</tr>
<tr>
<td>R.T.</td>
<td>Reverse Transcriptase</td>
</tr>
<tr>
<td>S.P.S.S.</td>
<td>Scientific Programme for Social Sciences</td>
</tr>
<tr>
<td>U/l</td>
<td>International Units</td>
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<tr>
<td>Z.D.V.</td>
<td>Zidovudine</td>
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ABSTRACT

Advanced HIV infection causes acquired immunodeficiency syndrome (AIDS) a condition in human in which the immune system is unable to fight off other diseases and other opportunistic infections. Upon entry, HIV infects vital cells in the human immune system such as helper T cells, also called CD4 cells, macrophages and dendritic cells leading to lower levels of these cells in the body. The virus lives and multiplies primarily in the white blood cells, the immune cells that normally protect one from diseases. The hallmark of HIV infection is the progressive loss of a specific type of immune cells called CD4 cells weakening the immune system and leaving the individual vulnerable to various opportunistic infections (OIs) and other illnesses, ranging from pneumonia to cancer. Complications such as hyperglycemia/ diabetes and pancreatitis have lately been associated with the onset of HIV infection and although documentation and studies on this relationship are not many, a few studies have indicated that 11 in every 100 HIV-positive individuals are diabetic and over 17% of HIV-positive have pancreatitis. The objective of this study was therefore to determine the prevalence of diabetes and pancreatitis by considering some diabetes risk factors, which included glucose metabolism and pancreas state in HIV infected individuals. Assessment of the state of glucose metabolism in the body was evaluated using the concentrations of blood glucose while pancreatitis was evaluated using the rate of amylase enzyme activity (amylase levels). The HIV infection was based on CD4 count and viral load. The study involved 193 participants who were grouped into two groups of 97 subjects of HIV negative individuals (also referred to as study control group) and 96 participants who were HIV-positive. In the study 13.54% of HIV infected individuals were hyperglycemic compared to 6.18% in HIV negative population with a mean blood glucose of 7.6±5.1 and 4.4±1.1 respectively, while the mean amylase level was 110.0±28.1 in HIV-positive individuals compared to 82.8±24.2 in the HIV negative group, the CD4 mean was 888.8±244.1 and 308.8±249.8 in the HIV negative group and in the HIV infected individuals respectively. The study showed significant negative correlation between amylase levels and CD4 count (P<0.05) (r= -0.451) and also a significant positive correlation between amylase levels and viral load (P<0.05) (r=0.697); this confirmed that elevated amylase level which is suggestive of pancreatitis results from advancement in HIV infection, a probable cause of abnormality in glucose metabolism in HIV positive individual. Although hyperglycemic was notable in HIV positive, there was no correlation between amylase levels and glucose levels (P>0.05) (r=0.311), there was also no correlation between blood sugar levels and CD4 counts (P>0.05) (r= -0.023) and between blood sugar and viral loads (P>0.05) (r=0.035). This may suggest that abnormality in glucose metabolism in HIV infected individuals is as a result of a complex interaction of many diabetes mellitus risk factors.
CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Human immunodeficiency virus infection occurs alongside other infections including diabetes. Diabetes and HIV have the same prognosis and the two diseases have similar symptoms which include weakening of immunity, reduction of body weight (body fat waste), and hyperinsulinemia with insulin resistance, glucose metabolism abnormalities, abnormal liver function with elevated activities of alanine amino transferases, neurovasculopathies with non-healing wounds, dementia, candidiasis and chest infections among others.

AIDS and diabetes are both independent diseases in that they are caused by different causative agents, AIDS is caused by the human immunodeficiency virus (retrovirus) that causes destruction of immune cells in the body leading to serious immune suppression and hence an onset of many other illnesses referred to as opportunistic infections (OIs). Diabetes on the other hand is a medical complication as a result of glucose metabolism abnormality with a diverse causative agents and it can be one of the complications of HIV infection.

Koeppe et al. (2006) suggested that uncontrolled HIV replication may cause diabetes mellitus in some patients; Similarly Gadd (2006) observed that the incidence of diabetes mellitus among HIV infected individuals was high compared
to the incidence in individuals not HIV infected. In this case an African man with advanced HIV infection had diabetes which resolved after viral replication was suppressed with antiretroviral therapy (ART)

1.2 Diabetes mellitus

Based on the pathogenesis diabetes can be classified as either type 1 or type 2. Type 1 is further sub-divided into two subtypes: immune mediated diabetes and idiopathic diabetes.

Immune-mediated diabetes results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such
individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis (Genuth et al., 2003).

In idiopathic diabetes, type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity (Genuth et al., 2003).

Type 2 diabetes (ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance). This form of diabetes which account for ~90–95% of those diabetic encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur (Inzucchi et al., 2009).

Other specific types of diabetes include; Genetic defects of the β-cell diabetes that is associated with monogenetic defects in β-cell function, genetic defects in insulin action diabetes that result from genetically determined abnormalities of insulin action, diseases of the exocrine pancreas diabetes which involves any
process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection; pancreatectomy, and pancreatic carcinoma.

Endocrinopathies diabetes—several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action, drug- or chemical-induced diabetes—Many drugs and toxins can impair insulin secretion they include Vacor (a rat poison) and intravenous pentamidine which can permanently destroy pancreatic β-cells, Infections diabetes is diabetes resulting from infection by certain viruses have been associated with β-cell destruction e.g. Congenital rubella, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the diabetes (Kitzmiller et al., 2003).

The long-term consequences of uncontrolled diabetes include neuropathy, retinopathy, and heart disease. It is also associated with vascular disease and is one of the major reasons for amputations (Boussageon, et al., 2006).

Insulin is a hormone that enables the body cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. The principal biochemical function of glucose is to provide energy for life processes. Glucose oxidation by the glycolytic and tricarboxylic acid pathways is the primary source of energy for the biosynthesis of ATP (Edelman et al., 2004).
1.2.1 Causes of diabetes mellitus

Independent risk factors in diabetes are old age, larger BMI category, liver damage and diseases and viral infections. In type 1 diabetes the pancreatic cells that make the insulin are destroyed causing severe lack of insulin (hypoinsulinemia). It is not clear what causes type 1 diabetes which account for about 5-10 % of diagnosed diabetes and develops most often in children and young adults, but the disorder can appear at any age, symptoms usually develop over a short period although beta cells destruction begin months, even years earlier. Symptoms appear when at least 80% of the cells are affected (Alemzadel et al., 2008).

The destruction of beta cells is thought to be the result of the body attacking and destroying its own cells in the pancreas, also known as autoimmune reaction. It is not clear why this happens, but a number of explanations and possibilities could trigger this reaction. These may include infection with a specific virus or bacteria (e.g. Cytomegalovirus whose induction of DM in mice has proven to be an excellent experimental model for the pathogenesis of viral diseases). Other include exposure to food-borne chemical toxins, disorder in the immune systems caused by virus infection whereby immune system cannot kill infectious agents or autoimmune systems response, as seen in type 1 diabetes mellitus, where by lymphocyte infiltration of the islet cells of the pancreas occur with concomitant beta cells destruction and the appearance of antibodies to islet cells components before the manifestation of diabetes (Tajima et al., 2002).
Other rare causes of diabetes (type 1 and 2) can include any other illness or disease that damages the pancreas elevating blood amylase levels and affecting its ability to produce insulin, e.g. pancreatitis, alteration of the hormone or cytokine levels, and interactions of the HIV's protein VPr with proteins responsible for glucose transport within cells (Jordan et al., 2006). Liver cirrhosis can cause insulin resistance and glucose intolerance (Selberg et al., 2003), a sign of developing diabetes and liver cirrhosis is common HIV infected individuals (Castellaras et al., 2008).

Depression a common problem in HIV infected persons may trigger the body turning on itself (autoimmune response), causing pancreatitis as in case of type 1 diabetes. More often HIV is accompanied by depression and patients with HIV infection, clinical depression is the most frequently observed psychiatric disorder affecting as many as 1 in 3 HIV infected people (Rabkin et al., 2002). Hardy (2006) observed that stress observed in HIV infected patients elicit a complex hormonal and immunological response that may alter various biochemical pathways, including glucose metabolism.

1.2.2 Clinical signs, symptoms and diagnostic criteria

Clinical signs for diabetes include polyuria, polydipsia, and blurred vision while thirst develops because of osmotic effects. Sufficiently high glucose in the blood is excreted by the kidneys, but this requires water to carry it and causes increased fluid loss, which must be replaced. A rarer but equally severe presentation is hyperosmolar non-ketonic state, which is more common in type 2 diabetes. Diabetes mellitus is also characterized by recurrent or persistent hyperglycemia, and
is diagnosed by demonstrating any one of the following (UNAIDS/WHO, 1999):

(i.) Fasting plasma glucose level at or above 7.0mmol/l. (ii.) Plasma glucose at or above 11.1 mmol/l two hours after glucose load in a glucose tolerance test. (iii.) Random plasma glucose at or above 11.1 mmol/l. In this study the DM diagnosis was based on random plasma glucose level estimation only.

1.3 HIV/AIDS

Advanced HIV infection causes acquired immunodeficiency syndrome a condition in human in which the immune system is unable to fight off other "opportunistic" infections, and other illnesses. Upon entry, HIV infects vital cells in the human immune system such as helper T cells, macrophages and dendritic cells leading to lower levels of these cells in the body. The virus lives and multiplies primarily in the white blood cells, the immune cells that normally protect the body from diseases. The hallmark of HIV infection is the progressive loss of a specific type of immune cells called CD4 cells also called T4 or T-helper cells, weakening the immune system and leaving the individual vulnerable to various OIs and other illnesses, ranging from pneumonia to cancer (Grabar et al., 2000; Kayabata et al., 2002).

The U.S. Centers for Disease Control and Prevention (2004) defines someone as having a clinical diagnosis of AIDS if they have tested positive for HIV and meet one or both of these conditions; they have experienced one or more AIDS-related infections or illnesses, the number of CD4 cells has reached or fallen below 200 per cubic millimeter of blood (a measurement known as T-cell count). In healthy
individuals, the CD4 count normally ranges from 450 to 1200 cell/mm$^3$. In some cases, the T-cells decline and OIs that signal AIDS develop soon after infection with HIV but in most cases it remains asymptomatic for 10 to 12 years, and a few for much longer. As with most diseases, early medical care can help prolong a person's life. Since the beginning of the epidemic, AIDS has killed more than 25 million people worldwide. AIDS has replaced malaria and tuberculosis as the world's deadliest infectious disease among adults and is the fourth leading cause of death worldwide.

HIV-infected individual carries the virus in the body fluids, including blood, semen, vaginal secretions, and breast milk. The virus can be transmitted only if such HIV-infected fluids enter the bloodstream of another person. This kind of direct entry can occur (1) through the linings of the vagina, rectum, mouth, and the openings at the tip of the penis; (2) intravenous injection with a syringe; or (3) through a break in the skin, such as a cut or sore. Usually, HIV is transmitted through sexual intercourse (AMFAR, 2006).

### 1.3.1 HIV diagnosis

According to WHO guidelines rapid HIV test kits are used in which two tests are done parallel. The rapid HIV test kits used included Determine, Unigold, and Bioline. The sensitivity and specificity of these kits is equal to ELISA and therefore when two serial or parallel tests are performed the results are confirmed. This is acceptable worldwide and whole blood, serum or plasma can be used (WHO, 2000)
1.4 HIV and Diabetes Mellitus

A relationship between HIV infection and diabetes onset has been suggested by several authors. For example a study by Aberg (2002), indicated a prevalence of diabetes of 2-7% among HIV infected individuals receiving protease inhibitors (PIs). The incidence of diabetes in HIV patients has been estimated to range from 1% to 10%. Carr et al. (2004) showed an increased incidence of insulin resistance among HIV +ve patients and hence a concern that this population in general and individual with evidence of fat redistribution common in HIV infected, in particular may be at increased risk of development of diabetes.

Edward (2006) suggested that type 1 diabetes occurs when islet of langerhan cells of the pancreas are destroyed probably as consequence of genetic susceptibility followed by the onset of autoimmune destruction triggered by environmental factors such as viral infection e.g. HI-virus, while Aberg (2002) showed that metabolic complications and disorders common with diabetics which include hyperlipidemia, insulin resistance and fat redistribution, are also common in HIV infected patients. It is therefore important to assess the difference in the diabetes prognosis between HIV infected and non-HIV infected individuals, the objective of this study was therefore to evaluate the difference in prevalence of diabetes in HIV infected and non HIV infected individuals and whether diabetes is a complication of HIV infection and also evaluated the existence of any relationship between HIV infection and diabetes onset.
1.5 Justification

Uncontrolled HIV infection result in many complications, which include other infections such as TB, cancer and metabolic disorders. This causes a major challenge in the management of the same which involves treating the complications first, for example treating for chest infections, before treating for the virus.

Onset of diabetes is also associated with a number of medical complications which would make the condition of a HIV victim even worse in the first place and complicate management of the HIV infection. At the same time symptoms such as weakened immunity, reduction of the body weight (body fat waste), hyperinsulinemia with insulin resistance, glucose metabolism abnormalities, abnormal liver function with elevated activities of alanine amino transferases, neurovasculopathies with non-healing wounds, dementia, candidiasis and chest infections, are common both in HIV +ve and diabetics. This can lead to clinical miss-diagnosis and miss-management of either of these diseases and especially because there are few documented studies on the relationship between HIV and diabetes.

A number of theories have been put forward to explain the increased occurrence of insulin resistance and diabetes in people with HIV. While the bulk of research have implicated proteases inhibitors (PIs), other explanations cannot be discounted, and it is likely that multiple factors are at play simultaneously.
This study therefore investigated the relationship between HIV and diabetes and also established the prevalence/incidence of diabetes in HIV infected individuals. This can help in management of HIV by factoring in diabetes mellitus risks.

1.5.1 Research questions

1. Are there differences in prevalence of diabetes in HIV infected and non-infected individuals?

2. Is diabetes one of the complications of HIV infection?

3. Do HIV +ve have elevated blood sugar levels as compared to the sugar levels in HIV –ve individuals?

1.5.2 Hypotheses

(i) Null-hypothesis

There are no differences in diabetes risk factors in HIV +ve individuals compared to HIV –ve individuals in Nyeri district.
1.6 Objectives

1.6.1 General objective

The broad objective was to evaluate the blood sugar level and amylase levels among HIV infected individuals in Nyeri district.

1.6.2 Specific Objectives

i) Determine the prevalence of diabetes in non HIV infected and in HIV positive individuals in Nyeri district.

ii) Determine the prevalence of pancreatitis in non HIV infected and in HIV positive individuals in Nyeri district.

iii) Determine the reference ranges for blood glucose levels, amylase levels, and CD4 count in the control group

iv) Determine mean levels of blood sugar, amylase and CD4 in HIV +ve and in non HIV infected individuals Nyeri district.

v) Determine correlations between the CD4 count, blood sugar levels, amylase activity and viral load in HIV positive individuals
CHAPTER TWO

MATERIALS AND METHODS

2.1 Study area

The study was carried out at the Nyeri Provincial General Hospital in Central province with a bed capacity of 320 of which 7% of these beds are occupied by HIV +ve patients.

2.2 Ethical considerations

A approval for the project was sought and granted by the Nyeri Provincial General Hospital. The participants were reassured of confidentiality in the handling of information and procedures involved in this study. Informed consent was sought from the participants and who were also requested to append their signature on the consent form in order to acknowledge their voluntary approval of participation.

2.3 Study design and population size

This was a systematic and cross-section study in which the 10th participant was picked at Nyeri provincial general hospital. The sample size was calculated using the formulae given by Fisher et al. (1983) method and defined by the equation;

\[ n = \frac{Z^2 pq}{d^2} \]
Where;

\[ n = \text{the desired sample size (when population is greater than 10,000)} \]

\[ z = \text{the standard normal deviation, usually set at 1.96 (or more simply at 2.0)} \]
which correspond to the 95% confidence level

\[ p = \text{the proportion in the target population estimated to have a particular} \]

characteristic. If there is no reasonable estimate, then 50% (0.5) is used

\[ q = 1.0 - p \]

\[ d = \text{Degree desired, usually set at .05 or .02 or .01.} \]

Using this equation \( N = 90 \) patients.

The study adjusted the number to 100 in order to complement for any error due to
chance variations. In the final data analysis, 96 HIV+ve, participants, and 97
healthy normal subjects were used.

2.3.1 Inclusion and exclusion criteria

All the individuals were asked to participate in the study on a voluntary basis after a
brief explanation of the aims and purpose of the study. Those who accepted to
participate were subjected to an interview using a questionnaire. The HIV infected
individuals living in Nyeri district for 6 months prior to the study, not on ARVs,
and with no history of DM previous to infection and not obese(participant with a
BMI > 30 were said to be obese) were included.
All HIV infected individuals who are not residents of Nyeri District, those on ARVs, those obese and those with DM History were excluded from the study.

For the healthy group, in addition to the above criteria, the subjects were excluded from the study if they had regularly consumed drugs with potential hepatotoxicity or nephrotoxicity (such as analgesics/anti-inflammatory agents, and aminoglycosides) and also those that could affect the blood sugar level and the other analytes involved in this study. Eligible subjects were then requested to sign a consent form provided. Only 193 study subjects met the criteria and were selected to participate in the study. They were categorized into two groups i.e. healthy and HIV positive group consisting of 97, 96 subjects respectively.

2.4 Blood sample collection

Six ml of venous blood sample was collected using a septic technique of which 2mls was put in “plain vial”. The blood was left to clot and then spun to obtain 1ml of serum which was frozen at -20°C (for amylase analysis), until the time of analysis. The other 2 ml was put in EDTA vacutainer for CD4 count and the remaining sample, about 2ml, was also put in EDTA vacutainer and was used for blood sugar analysis and then spun to obtain about 1ml of plasma. The plasma was for viral load analysis and it was stored at-20°C. The containers were labeled with the study number of the participant and the date of birth was also marked to tally with all the required demographic information. This was matched with the demographic information on the questionnaire form to avoid any risk of mix-ups or incorrect identification of samples.
2.5 Biochemical analysis

2.5.1 Glucose levels determination by use of glucose oxidase method

In this method the aldehyde group of glucose is oxidized by glucose oxidase to give gluconic acid with the glucolactone as intermediate and hydrogen peroxide, in presence of peroxidase and oxygen acceptor a coloured compound is formed whose intensity is read spectrometrically.

Procedure:

Requirements include photometer which should be powered for 10min. before the test and set wavelength at 500nm, 5 tubes and micropipette/tips, these 5 tubes were labeled as the blank, std, control1, control2 and pt 1,2,3 etc and in each tube1.0ml of reagent put. Using micropipette, changing tips between samples 10uL of the sample was added. The mixture was then mixed by inversion after the tubes were capped and incubated for 10min at 37°c then after the incubation absorbance was read and the results calculated.

Results calculation formula

Sample Abs-blank Abs X glucose std. conc. = Sample conc. mmol/L.
Abs-Blank Absl (Human liquicolor kit manual, 2006).
2.5.2 CD4 determination

The FACSCOUNT™ machine was used to determine the CD4 cells count where fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens and a fluorescent nucleus dye binds to the nucleated blood cells. After a fixative solution is added to the reagent tube, the cells come in contact with the laser light, which cause fluorochrome labeled cells to fluoresce; this fluorescent light provides the information necessary for the instrument to count the cells.

**Procedure:**

The requirements included FACS count machine, CD4 reagents, coring station vortexer, 50ul pipette, fixative solution and whole blood collected in K2EDTA/K3EDTA anticoagulant. The tap of reagent was labeled with participant number e.g. 1, 2, 3, etc. Reagent was first vortex upright and then upside down for 5 seconds and the participant whole blood was also mixed by inverting the tube 8 times. 50ul of the sample was put into the reagents tube and then the tubes were capped and vortex for 5 seconds. This mixture was incubated for 60-120 minutes (20° – 25°C) and then 50ul of fixative was put into each reagent tube and mixture vortex for 5 seconds, finally the tubes were run on FACSCOUNT™ machine in which about 20ul sample was sucked through the machine probe and the cell count recorded as cells/ml of blood. These beads function as fluorescence standard for locating the lymphocytes and also a quantitation standard for enumerating the cells. (BD Biosciences .com Asia pacific).
2.5.3 Viral Load determination

The requirements included: 2ml sample of serum, ExaVir load-Quantitative Determination version 2 machine™ whose set up include; the high sensitive poly A plate, the RT reaction components that contains lyophilized odT and the RT product tracer that contains lyophilized monoclonal α-BrdUMP The principle of the test is that the lysates contains reverse Transcriptase activity (RT) enzyme will synthesis a DNA-strand whose product is detected with alkaline phosphatase (AP) conjugate alpha BrdU anti body. The product is then quantified by addition of a colorimetric AP substrate on the polyA plate and read calorimetrically at A405 as the number of the cells/μL (Cavidi Tech AB, Uppsala –Sweden) Roche Cobas Mira analyzer-Photometer. The Exavir (Exavir load Version 2 Quantitative determination of reverse transcriptase activity, 2004).

Procedure

Plasma prepared from EDTA anti-coagulant whole blood was used and it was first frozen and after thawing at or below 37°C in the collect and thaw component, 1ml plasma sample was sucked into ExaVir load-Quantitative Determination version 2 Machine™ and the measurement taken and recorded automatically.

2.5.4 Amylase activity determination

Alpha amylase was determined by Aeroset system operation (ASO). Alpha amylase hydrolyses the 2 chloro-4-nitrophenyl alpha- D maltotrioside to release 2-c-4 nitrophenyl alpha D maltoside and glucose. The rate of formation of 2-c-4nitrophenol is determined spectrophometrically at 404 nm as direct measurement
of alpha A activity (ASO manual 2007). 2 μl of 3 diluted samples was run on an autoanalyser machine and amylase activity levels recorded in U/l and then compared with reference ranges for adults.

Procedure:
The reagents MES buffer, pH 6.0±0.1, 2 chloro-4 nitrophenal alpha-D- maltotriside is provided as ready to use liquid and stored at 2-8°C. Other requirements include accurate pipettes, timer, and water bath at 37°C and spectrophotometer at 405nm. Reagent is first brought to room temperature and 1.0ml of the reagent is pipette into the tubes labeled control and test which were first Pre-incubate at 37°C for 5min. 0.025ml of the test and control samples were added into the respective tube and read spectrophotomectally other readings every 60 sec. for 2 min are done and the mean absorbance difference per min is taken i.e. ΔAbs/min.

Results Calculation:
ΔAbs/min X 3178 to obtain results in U/L

2.6 Quality control assessment
Quality control assessment (QCA)) was regularly carried out to ensure that the results obtained were accurate and reliable. It involved the total check on the personnel, equipment, reagent, specimen and analytical methodology. To carry out this QCA, a quality control material provided by the manufacturer of the various kits was always run in parallel with the test samples. Through the internal quality control (IQC), the quality of the result was checked. This helped in the detection
and rectification of various procedural processes that play a part in error creation. The validity of the reactions was monitored by use of these control sera with known normal and abnormal values. These controls were run once in every working shift.

2.7 Data analysis

Statistical analysis was carried out using SPSS program version 11.0. The data obtained was parametric in nature and was tabulated as mean and standard deviation. Means differences between the groups i.e. CD4 count between healthy and HIV +ve individuals, blood glucose levels between healthy and HIV+ve, amylase activity between healthy and HIV+ve individuals and viral load count in HIV+ve were assessed by Anova and post Anova statistical analysis. Pearson’s correlation coefficients (r) were calculated to determine relationship between means of the studied markers. Results were considered statistically significant at $p < 0.05$. The reference ranges for the various markers were calculated using the normal healthy individuals (reference group).
CHAPTER THREE
RESULTS

3.1 Demographic data

The age bracket of the control group (42 males and 58 females) was 15-44 years, with a mean of $41.11 \pm 12.15$ years. The HIV+ve patients consisted of 100 subjects (18 males and 82 females) attending Comprehensive Care Centre (CCC) PGH-Nyeri whose age ranged from 22-45 years, with a mean of $41.97 \pm 13.28$ years.

3.2 The prevalence of hyperglycemia in the HIV -ve population and in HIV positive participants

The prevalence of hyperglycemia in the healthy population was 6.28% and (with a glucose concentration mean of $4.4 \pm 1.1$) while in the HIV positive individuals was 13.54%, (with glucose concentration mean of $7.6 \pm 5.1$). This was more than double that of the HIV-ve population (Table 3).

Table 1: The prevalence of hyperglycemia-diabetes in control and in HIV positive participants

<table>
<thead>
<tr>
<th></th>
<th>Diabetics</th>
<th>Not diabetics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-negative</td>
<td>6</td>
<td>90</td>
<td>97</td>
</tr>
<tr>
<td>HIV-positive</td>
<td>13</td>
<td>83</td>
<td>96</td>
</tr>
</tbody>
</table>
3.3 The prevalence of elevated amylase levels in control group and in HIV positive participants

The prevalence of elevated amylase levels (i.e. > 150U/L ) in non HIV positive participants was 1.0% (with a mean of amylase Level mean of 82.8±24.2), this was about 5 times less than the prevalence of elevated amylase levels in HIV positive participants which was 5.2% (with a mean level of 110.0 ± 28.1).

Table 2: The prevalence of elevated amylase levels in control group and in HIV positive participants

<table>
<thead>
<tr>
<th></th>
<th>Elevated Amylase</th>
<th>Normal Amylase</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-negative</td>
<td>1</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>HIV-positive</td>
<td>5</td>
<td>91</td>
<td>96</td>
</tr>
</tbody>
</table>

3.4 Reference ranges for blood glucose levels, amylase levels, and CD4 count in the control group

Table 3 shows the mean blood sugar levels, amylase levels and CD4 count in normal healthy individuals (control study group). There were no significant differences in the mean blood sugar levels, amylase activity levels and CD4 count between the males and females ($p>0.05$). Therefore, the reference ranges for the blood sugar levels, amylase levels and CD4 count were determined using combined data for males and females.
Table 4 shows the combined mean blood sugar levels, amylase levels and CD4 count for the both males and females and the developed reference ranges for the blood sugar level, amylase activity level and CD4 count. The lower and upper reference range limits for blood sugar levels were 3.2 and 7.5 Mmol/l respectively, while the amylase activity levels were 35.9 and 130.4U/l respectively and CD4 count were 410.3 and 1367.2 cells/ml respectively.

Table 3: Reference ranges for blood glucose level, amylase activity level, and CD4 count in the study control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>X±SD</th>
<th>1.96SD</th>
<th>Ref Ranges</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>M</td>
<td>41</td>
<td>4.3±1.1</td>
<td>2.17</td>
<td>2.13-6.5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>54</td>
<td>4.5±1.0</td>
<td>1.96</td>
<td>2.54-6.5</td>
</tr>
<tr>
<td>CD4</td>
<td>M</td>
<td>41</td>
<td>865.8±225.7</td>
<td>442.31</td>
<td>423.49-1308.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>54</td>
<td>905.6±257.5</td>
<td>504.61</td>
<td>401-1410.2</td>
</tr>
<tr>
<td>Amylase</td>
<td>M</td>
<td>41</td>
<td>86.0±23.5</td>
<td>46.06</td>
<td>39.94-132.1</td>
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<tr>
<td></td>
<td>F</td>
<td>54</td>
<td>80.3±24.7</td>
<td>48.37</td>
<td>31.92-128.7</td>
</tr>
</tbody>
</table>
Table 4: Mean reference values for combined males and females

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>X±SD</th>
<th>1.96 SD</th>
<th>Ref Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>95</td>
<td>4.4±1.1</td>
<td>2.16</td>
<td>2.24-6.56</td>
</tr>
<tr>
<td>CD4</td>
<td>95</td>
<td>888.8±244.1</td>
<td>478.4</td>
<td>410.3-1367.2</td>
</tr>
<tr>
<td>Amylase</td>
<td>95</td>
<td>82.8±24.2</td>
<td>47.4</td>
<td>35.9-130.4</td>
</tr>
</tbody>
</table>

3.5 The mean blood sugar levels, amylase level, and CD4 count in HIV-ve and HIV+ve groups

Table 4 shows the mean blood sugar level, amylase level and CD4 count in HIV-ve and HIV+ve individuals. The mean blood sugar level in the healthy individuals was 4.4 ± 1.1 Mmol/l while in the HIV+ve was 7.6 ± 5.1 Mmol/l. The mean amylase activity in the normal healthy individuals was 82.8 ± 24.2 U/l compared to 110.0 ± 28.1 U/l in the HIV+ve group. The mean of CD4 count in the normal individuals was 888.8 ± 244.1 cells/ml compared to the HIV+ve which was 308.8 ± 249.8 cells/ml. These were significantly different (p<0.05).
Table 5. Mean levels of the measured parameters in the Control and HIV-positive individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=95) (HIV-ve)</th>
<th>HIV Positive Subjects (n=97)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (Mmol/l)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>4.4 ± 1.1</td>
<td>7.6 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>82.8 ± 24.2</td>
<td>110.0 ± 28.1</td>
<td>0.02</td>
</tr>
<tr>
<td>CD4 Count (Cells/ml)</td>
<td>888.8 ± 244.1</td>
<td>308.8 ± 249.8</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Figure 1: Mean glucose levels in control and HIV-positive individuals

Figure 2: Mean amylase levels in control and HIV-positive individuals

Figure 3: Mean CD4 count in control and HIV-positive individuals
3.6 Correlation of levels of blood sugar, amylase activity, CD4 count, and viral load in HIV+ve participants

Table 6 shows Pearson’s correlation coefficient \((r)\) between the measured parameters of blood sugar level, amylase activity, CD4 count, and the viral load in the HIV positive participants. There were negative significant correlations between the CD4 count and the viral load \((r= -0.697; p<0.05)\) whereby an increase in every 100 CD4 cells corresponded to a decrease in 305 viral particles, in HIV positive participants, the correlations was not significant between viral load and blood sugar \((r= 0.034; p>0.05)\), in HIV positive participants, correlations between blood sugar and CD4 count was also not significant \((r= -0.0235; p>0.05)\) in HIV positive participants. The correlation between CD4 and amylase levels was negatively significant \((r= -0.451; p<0.05)\) where-by an increase of 56 CD4 cells brought about 15 units decrease in amylase level in HIV positive participants. The correlations between the viral load and the serum amylase levels in HIV positive participants was positively significant \((r= 0.697; p<0.05)\) where an increase of 100 viral particles brought about two and half increase in amylase level, and finally the correlations between serum amylase and blood sugar was not significant \((r= 0.311; p>0.05)\) in HIV positive participants.
Table 6 Pearson’s correlation coefficient ($r$) between all the measured parameters

<table>
<thead>
<tr>
<th>parameters</th>
<th>r</th>
<th>p</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar vs amylase</td>
<td>0.311</td>
<td>0.752</td>
<td>No significant +ve correlation</td>
</tr>
<tr>
<td>Sugar vs CD4</td>
<td>-0.023</td>
<td>0.826</td>
<td>No significant correlation</td>
</tr>
<tr>
<td>Sugar vs Viral load</td>
<td>0.035</td>
<td>0.733</td>
<td>No significant correlation</td>
</tr>
<tr>
<td>Amylase vs CD4</td>
<td>-0.451</td>
<td>0.000</td>
<td>Significant –ve correlation</td>
</tr>
<tr>
<td>Amylase vs viral load</td>
<td>0.697</td>
<td>0.000</td>
<td>Significant +ve correlation</td>
</tr>
<tr>
<td>CD4 vs Viral load</td>
<td>-0.678</td>
<td>0.000</td>
<td>Significant –ve correlation</td>
</tr>
</tbody>
</table>
CHAPTER FOUR

DISCUSSION, CONCLUSIONS AND RECOMMENDATION

4.1 Discussion

There are very few studies on the status of diabetes or even on the relationship between HIV infection and diabetes. This relationship was first proposed by Gadd (2006) who suggested that elevated blood sugar levels could be a complication of untreated HIV infection.

In Kenya there is limited literature on the prevalence of diabetes among HIV infected despite the diseases prevalence in the general population having been on the rise (Jalang'o, 2006). HIV infection still remained significantly high with a prevalence estimated at 7% nationally (NASCOP, 2004). There are some studies that have observed the existence of relationship between HIV infection and diabetes (Aberg, 2002; Currier et al., 2002; Gadd, 2006).

The main objective of this study was to compare the prevalence of diabetes, measured as elevated random blood sugar, among HIV and non-HIV infected individuals. The study investigated hyperglycemia and the possibility of pancreatitis in relationship to advancement of HIV infection where by both CD4 count and viral load were determined in HIV +ve and the mean blood sugar and amylase level
determined. The correlation between the different parameters here tested and corrected as Pearson’s correlation coefficient.

This study found that the prevalence of diabetes mellitus in the study group was similar to national prevalence of 6.7% (average for both Rural and urban population) (Menya, 2010), this was likely because the study was dealing with the same population.

The prevalence of hyperglycemia in HIV positive was more than double that of the control group. At the same time, levels of amylase were found to be elevated (Elevated amylase level have been associated with pancreatitis) in HIV infection, while amylase levels in the control group was within normal ranges, suggesting a relationship between pancreatitis and HIV infection. This is similar to finding by other studies that have indicated that pancreatitis is common in HIV infection (Miller et al., 1992). Among the common causes of pancreatitis are autoimmune reaction of the pancreas cells (Hardy, 2006) triggered by exposure to food borne chemical toxins, and viral infection (Tajima et al., 2004). Type 1 diabetes has been suggested to occur when islet of langerhan cells of the pancreas are destroyed (pancreatitis) (Edward, 2006), causing insulinepenia (insufficient insulin secretion) and hence the body cells are unable to absorb glucose from the blood (Christopher et al., 2006). In conclusion, therefore, the elevated amylase levels and subsequently hyperglycaemia, that this study observed, may be a complication of HIV infection.

This study also determined the reference ranges for blood glucose, amylase enzyme and CD4 count in the HIV-ve population (control group). There were no significant
differences in the established reference ranges between the males and the females \((P>0.05)\) and therefore they were combined together to obtain a common reference range. The reference range for blood glucose levels, amylase levels and CD4 count for the study group differed from the ranges given by the kit manufacturers and in other studies. The blood glucose reference ranges in this study was found to be slightly lower than that in other studies (Bimenya et al. (2006), the same case with amylase levels (Owyang et al. (2007), while the CD4 was completely different from that of Partec CD4 easy count Kit. The above differences can be explained by the fact that the sample size used in this study is just a small one as oppose to the thousands used by manufacturers to develop reference ranges, and also the fact that these kits are manufactured in the western countries and the study populations used in the development of these reference ranges are of different race and different geographical distribution from our local population and therefore because of genetical and geographical differences there is need to develop our local reference ranges as opposed to using ranges given by the manufacturers especially if they are from western countries.

The study also established means blood sugar levels, amylase levels, and CD4 count in the control group and HIV positive participants in which case the means in the above parameters was found to be different and the differences was found to be significant \((p<0.05)\). The mean blood sugar level in the HIV +ve group was found to be higher than that of the control group (almost double that of the control group) this is suggestive of hyperglycaemia in HIV positive participants; this is similar to observations by other studies. Uncontrolled HIV replication may be the cause of
diabetes mellitus (DM) in some patients (Currier et al., 1999 and Koeppe et al., 2004), therefore the incidence of DM among human immunodeficiency virus (HIV) infected individuals is high compared to the incidence of DM in individuals not infected with HIV (Gadd, 2006). This can explain why increased levels of insulin (hyperinsulinemia) are required to maintain normal blood sugar level in HIV positive individuals (Kawabata et al., 2002).

The means amylase levels in the HIV-ve individuals was found to be similar to that observed by other studies and termed normal (Owyang, 2007), while in HIV +ve individuals it was found to be elevated, this can be suggestive of pancreatitis in HIV infected individuals and this agrees with a study that observed 17% of children with HIV suffer from pancreatitis and this prevalence rate is even higher in adults (Miller et al., 1992). Pancreatitis in HIV infection can be caused by cancer and viral infection like cytomegalovirus 4 cryptosporidium common OIs in HIV positive victims (Giuseppe et al., 1997).

There was significant difference between the mean CD4 count in the control group and the HIV +ve individuals. This difference is due to progressive loss of these specific types of immune cells called CD4 cells also called T4 or T-helper cells, weakening the immune system of HIV infected and leaving the individual vulnerable to various opportunistic infections and other illnesses that range from pneumonia to cancer (Kayabata et al., 2002; Sowarsky et al., 1999). CD4 count (i.e. the number of CD4 cells per micro liter (μl) of blood) is the standard laboratory test for assessing HIV stage and prognosis and for monitoring progression to AIDS and risk of opportunistic illness. It is also used as guide on when to initiate antiretroviral
treatment (ART), and beginning prophylaxis for opportunistic infection. The CD4 count typically declines at the rate of about 30% per day in patients with CD4 count of less than 500 cells/mm$^3$ as HIV infection progresses (Neumann et al., 1995).

The other objective of this study was also to determine correlations between parameters of CD4 count, blood sugar levels, amylase activity and viral load in HIV positive individuals. The study found the correlation between CD4 count and viral load in HIV positive groups significantly negative, which indicated active HIV infection (Hogg et al., 2001).

The correlations between the viral load and the serum amylase levels were significantly positive while the correlations between CD4 and amylase levels were found to be significantly negative. These observations suggested that elevated amylase activity which is indicative of pancreatitis occurs in advanced HIV infection and this agrees with other studies that have observed that pancreatitis in advanced HIV infection resulting into islet of langerhan cells of the pancreas destruction. This probably results from genetic susceptibility followed by the onset of autoimmune destruction triggered by environmental factors such as viral infection e.g. HI-virus, and other opportunistic infections (Edward, 2006).

There were no correlation between blood sugar level and other parameters like CD4 count, viral load, amylase levels, this is a picture of what has been referred to as 'compensated normoglycemia' (Carr, 1998) which results from of insulin resistance. Insulin resistance in HIV infection is associated with HIV lipodystrophy
syndrome, a syndrome that is not very well understood but result to Changes in fat redistribution (increased visceral adiposity and reduced subcutaneous fat) which is severe in HIV positive patients and may contribute independently to hyperlipidemia and insulin resistance (Colleen et al., 2001).

4.2 Conclusion

In conclusion, the present study demonstrated that:

1. Hyperglycemia was a major complication in HIV infection as characterized by increased blood sugar levels.

2. The mean amylase level among HIV infected was higher than that in healthy individuals (110±28.1, 82.8±24.2 respectively), suggesting that pancreatitis is a likely complication in HIV infection.

3. There was a strong relationship between HIV infection and elevated amylase levels.

From these conclusions the null-hypothesis, stating that there are no differences in prevalence of diabetes in HIV+ve compared to HIV-ve individuals in Nyeri district, was consequently rejected, and the alternate- hypothesis stating that there is difference in the prevalence of diabetes in HIV+ve individuals compared to HIV-ve individuals in Nyeri district was accepted.
4.3 Recommendations

- Understanding the glucose metabolism disturbances that are possible with HIV infection on-set is important and therefore performing appropriate screening for glucose intolerance and diabetes are recommended as components for care for HIV patients.

- It is recommended that progressive tests on the damage of the pancreas (pancreatitis) be regularly done in HIV patient.

- It is recommended that perspective studies to ascertain glucose metabolism disturbances and damage of pancreas with the onset of HIV infection to be done.
REFERENCES


Kovacs, J.A. (2001). Identification of dynamically distinct subpopulation of T lymphocytes that are differentially affected by HIV. Medical Express, 194: 1731-1741.


APPENDICES

Appendix 1: Explanation and consent form

The risk factors of diabetes in HIV infected individuals based on blood sugar level and amylase activity level.

Information sheet on ethical issues of the study

a) Information to the subjects

I am doing a study on "the risk factors of diabetes in HIV infected individuals" in this District. To be eligible to participate in this study, you should preferably be 15 years of age and resident of this District. You will be requested to give consent to this study at your will. If you give consent, you will be asked some questions and given a form to sign. You will be requested to give 5ml of blood. Your consent will give me access to your information in the Hospital file. However, this information will be used for this study only. It will be confidential. Your name will not appear anywhere except in the consent form and the questionnaire. The final results will not show your name. Further, your information will not be revealed to any one else without your consent. You are under no pressure or any obligation to consent this study. You are encouraged to ask any question you may be having before signing the consent and in a language you understand. If you need to consult before making this decision, you are free to do so.

b) Purpose of the Study

The study will investigate whether HIV infection in predisposing you to risks of becoming diabetic due immune suppression. The tests we are going to carry out...
include determination of your HIV stature and blood sugar level, amylase activity attest aimed at determining the effect of HIV infection to the pancreas. We are also going to determine your CD4 count and your viral load. Routinely, CD4 count and viral load are done to determine the advancement or the stage of HIV infection but this has not been related to the damage on the pancreas or abnormalities in the glucose metabolism. This will go a long way in reducing cases of diabetes developing with the onset of HIV infection and also improve HIV complications management.

c) Patient’s Responsibility

After willingly enrolling for the study, you will be requested to give 5ml blood to me, which will be taken to the laboratory for the analysis. You will be requested to give authority to the use of your information in the Hospital file such as the type of medication you are on and any other medical condition that is relevant to this study.

d) Risks

During sample collection, you will feel some pain. This will be minimal. A scar is likely to form although not always. Personal information in this study will be kept confidential. Study forms will be kept under lock and key to which no one else except me will have access. The study forms will not be identified with your name but only with a study number.

e) Benefits

If you are found to have hyperglycemia you will be screened/confirmed for diabetes, you will be treated and given appropriate medical advice. This study will allow us to make a diagnosis and appropriate management.
f) Other pertinent information

i). You will not be required to pay anything for the estimation of the various parameters, which will include blood sugar level and diabetes test, amylase activity determination, CD4 count and viral load.

ii). The study will be voluntary. You may refuse or withdraw from the study at any time without penalty, intimidation, threat or loss of benefits you are entitled.

iii). The monitor, the auditor, ERC and the regulatory authority will be granted direct access to the study.

iv). Your identity will not be revealed. Codes and numbers will be used. When the results will be published, your identity will remain confidential.

Before I involve you in the study, I kindly request you to append your signature below in the consent form.

I................................................................. have read and understood the purpose and benefits of the study and I hereby agree to participate in the study.

Participant's signature.................................Date.................
Appendix 2

FACSCount CD4/3 SW Ver 1.0 08/05

Control Results
KEMRI
Operator : AOA

Control Bead Lot ID : 09061721
Control Bead Counts - beads/ul
  low : 47  med : 239  high : 962

Reagent Lot ID : 00272121
Reference Bead Counts - beads/ul
CD4 Tube : 1013

Date : 8/15/09 16:18
Control Run : PASSED
Lab Normal ID: 00

Absolute Counts - cells/ul:

<table>
<thead>
<tr>
<th></th>
<th>CD4</th>
<th>CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>low tube</td>
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<td>745</td>
</tr>
<tr>
<td>medium tube</td>
<td>441</td>
<td>649</td>
</tr>
<tr>
<td>high tube</td>
<td>482</td>
<td>703</td>
</tr>
</tbody>
</table>

Mean Counts : 481 699
Range : 81 96

Absolute Counts - beads/ul:

<table>
<thead>
<tr>
<th></th>
<th>low tube</th>
<th>medium tube</th>
<th>high tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52</td>
<td>285</td>
<td>1034</td>
</tr>
</tbody>
</table>

Control Bead Results:
R : 0.9996
Slope : 1.06
Intercept : 15

Reference Bead Cluster Locations:

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<tr>
<th></th>
<th>low tube</th>
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</tr>
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<td>226,224</td>
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Signature: ________________________________

Comments: ________________________________

Instrument Warnings:

Code 29. Check system fluid tank level
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<tr>
<th>DATE</th>
<th>Absolute counts</th>
<th>casets/ul</th>
<th>Absolute counts beads cells/ul</th>
<th>Control Beads Results</th>
<th>Refl Bead Cluster locations</th>
<th>Comments</th>
<th>Initials</th>
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<td></td>
<td>low tube</td>
<td>medium</td>
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**Appendix 3**

**Comments:**

**QCQA Review:**

_20/10/07_
| DAILY ACTIVITY                                      | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---------------------------------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---- |
| Clean bench with 5% JIK                           |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| Morning                                           |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Evening                                           |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| Chart Temperature                                 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Check Rgts and supplies                           |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| Waste segregation done                            |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| QC done for all tests LMH                         |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Fill daily maintenance logs                       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Check blood pints for Exp & FEFO                  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Log in Results                                    |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| All results sent to Dispatches (EVE)              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Wiped spillages with 1% JIK                       |   |   |   |   |   |   |   |   |   | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ | √√ |
| Decontaminate reusable items                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| with 1% JIK                                       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Techs initials                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

COMMENTS: Checked 20/6/09

REVIEW:

NB: USE A TICK TO INDICATE PERFORMANCE
KEY: NA=Not Applicable, WKND=Weekend, HOL=H=NO, Y=YES, P=PARTIAL; EVE=EVENING

NB: PARTIAL APPLIES TO RAPID TESTS

VERSION # 2   SOP #   EPGH

NYERI PGH LABORATORY
DAILY ACTIVITY LOG
DEPARTMENT/LOCATION CDH MONTH JUNE YEAR 2009
Appendix 5: Questionnaire

THE RISKS FACTORS OF DIABETES MELLITUS.

STUDY NUMBER

CASE

1. SEX

MALE FEMALE

2. AGE. YRS

3. DISTRICT OF BIRTH

NYERI OTHERS

4. IN THE LAST 6 MONTHS HAVE BEEN RESIDING IN NYERI

YES NO

5. ARE YOU DIABETIC

YES NO

IF YES WHEN WERE YOU DIAGNOSIZED WITH DIABETES FOR THE 1ST TIME ...............
6. DO HAVE A CLOSE FAMILY MEMBER WHO IS DIABETIC

- **YES**
- **NO**

If yes, who...

7. HIV STATUS

- HIV+VE
- HIV-VE

8. ARE YOU TAKING ANY ART

- **YES**
- **NO**

If yes, which one...

9. ARE YOU TAKING ANY DRUG

- **YES**
- **NO**

If yes, which one...

10. ARE YOU TAKING ANY DIABETIC MEDICATION

- **YES**
- **NO**

If yes, which one...

11. DO YOU SMOKE

- **YES**
- **NO**

12. DO YOU TAKE ALCOHOL

- **YES**
- **NO**
Appendix 6: Screening Form for Controls

The risk factors of diabetes mellitus

STUDY CASE NUMBER.................................................................

SEX........................................DATE OF BIRTH..............................

DO YOU HAVE ANY MEDICAL PROBLEM?...............................YES/NO

IF YES, WHICH ONE (S)...............................................................}

...............................................................YES/NO

DO YOU HAVE A MEMBER(s) OF YOUR FAMILY WHO SUFFER FROM DIABETES.....YES/NO

IF YES WHO (e.g. My Father etc)..............................................................

URINALYSIS

PH.................................................................

S.G.................................................................

PROTEIN...............................................................POSITIVE/NEGATIVE

BLOOD...............................................................POSITIVE/NEGATIVE

KETONES...............................................................POSITIVE/NEGATIVE

NITRITES...............................................................POSITIVE/NEGATIVE

BILIRUBIN...............................................................POSITIVE/NEGATIVE

UROBILINOGEN...............................................................POSITIVE/NEGATIVE

LEUCOCYTES...............................................................POSITIVE/NEGATIVE

GLUCOSE ...............................................................POSITIVE/NEGATIVE