THE POTENTIAL FOR DIPEPTIDYL PEPTIDASE IV (DPPIV) USAGE AS A SURROGATE MARKER FOR ANTIRETROVIRAL THERAPY EFFICACY IN HIV INFECTED POPULATIONS

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The Potential for Dipeptidyl Peptidase IV (DPPIV) Usage as a Surrogate Marker for Antiretroviral Therapy Efficacy in HIV Infected Populations

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2017
DECLARATION

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

Signature……………………………………… Date…………………………

Ayub Kiprotich Maina

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

Signature……………………………………… Date…………………………

Prof. Elijah Maritim Songok, PhD

KEMRI, Kenya

Signature……………………………………… Date…………………………

Prof. Daniel Kariuki, PhD

JKUAT, Kenya
DEDICATION

I dedicate this thesis to the soul of my departed brother, Phillip Towett who I lost at the beginning of this work. His insights and advice about life inspired me to do this work. I also dedicate it to my mother Hellen Laigong whose overwhelming support carried me throughout the implementation of this work.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ART</td>
<td>Anti-retroviral therapy</td>
</tr>
<tr>
<td>ARVs</td>
<td>Antiretroviral drugs</td>
</tr>
<tr>
<td>ADA</td>
<td>Adenosine Deaminase</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of Differentiation</td>
</tr>
<tr>
<td>DPPIV</td>
<td>Dipeptidyl peptidase IV</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>TDF-</td>
<td>Tenofovir Disoproxil Fumarate</td>
</tr>
<tr>
<td>NVP-</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>LPV/r-</td>
<td>Lopinavir/Ritonavir also called Kaletra</td>
</tr>
<tr>
<td>AZT-</td>
<td>Azidothymidine also called zidovudine</td>
</tr>
<tr>
<td>3TC-</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>ABC-</td>
<td>Abacavir</td>
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ABSTRACT

The most commonly used markers for monitoring efficacy of anti-retroviral therapy in Human Immunodeficiency Virus (HIV) infected individuals are the viral load and CD4+ cell counts. Viral load monitoring is limited in resource limited countries due to its high cost, therefore leaving the use of CD4+ T cell counts as the only alternative for evaluating HIV infected individuals. CD4+ cell counts is an unreliable predictor of disease progression even though it is cheaper and most readily available in these settings. There is therefore a need to develop more sensitive and less costly alternative techniques for the detection of treatment failure which can be of utmost importance in these resource limited settings. This study sought to evaluate the feasibility of using Dipeptidyl peptidase IV (DPPIV) in plasma as a novel marker for the clinical evaluation of efficacy of treatment in young HIV infected individuals. DPPIV is an enzyme that cleaves N-terminal dipeptides next to an alanine or proline residues. Blood samples were collected from HIV positive young individuals (n=76) before and after initiation of ART, then assessed for HIV RNA (viral load), CD4+ T cell count and DPPIV levels. Roche Amplicor HIV-1 Monitor Test kit was used to analyse viral load levels while the BD FACS Calibur flowcytometer was used to analyse the levels of CD 4+ T cell counts with the Human DPPIV Quantikine ELISA kit (R&D Systems, Minneapolis MN) used to analyse DPPIV levels. The levels of plasma DPPIV increased significantly in study participants after ART initiation (p = 0.017), while the levels of viral load declined after ART initiation with an increase in CD4+ cell counts. There was a weak correlation (r=0.26) between the change in DPPIV after ART to the change in viral load after ART while there was a no correlation (r=0.14) between the change in DPPIV levels after ART to the the change in CD 4+ cell counts after ART. There was no statistically significant difference in DPPIV (p=0.7460), viral load (p=0.9875), and CD 4+ cell counts (p=0.548) with gender. The findings further showed a significant inverse relationship between the DPPIV levels (p=0.0017 increase after ART) and HIV viral load levels.
(p=0.0001 decrease after ART) and the presence of a direct relationship of DPPIV levels (p=0.0017 increase after ART) to the CD4+ Cell counts levels (p=0.0001 increase after ART). The latter suggesting a potential for DPPIV use as a more cost effective and a sensitive alternate surrogate marker for the evaluation of HIV disease progression in young individuals on HIV treatment.
CHAPTER ONE

INTRODUCTION

1.1 Background

Since its emergence, HIV/AIDS has remained as one of the worst scourges to afflict humanity (Gottlieb et al., 1981; Barre-Sinoussi et al., 1983; Schupbach et al., 1985; Gallo et al., 2006). By the end of 2014, WHO estimated the number of people living with HIV/AIDS at 36.3 (35.3-39) million people globally, and the number of those who had died from AIDS related illness since 1981 at 39 million (UNAIDS, 2013). The development of anti-retroviral (ARV) drugs has been one of the most significant milestones in the battle against HIV/AIDS, and has largely been responsible for significant reduction in morbidity and mortality among infected individuals. In recent years, the numbers of annual AIDS-related deaths have decreased from a global peak of 2.1 million in 2004, to an estimated 1.2 million in 2016 (UNAIDS, 2015). This has been largely attributed to increased availability and coverage of ART, care and support of people living with HIV/AIDS (UNAIDS, 2013).

Antiretroviral drugs (ARVs) are classified depending on the stage of the virus life cycle they target, with most of the drugs categorized based on their enzymatic targets [Wainberg and Jeang, 2008]. The first class of ARVs to be developed, targeted the viral enzyme reverse transcriptase, and subsequently new drugs targeting viral protease, integrase and HIV fusion proteins have been developed, increasing the treatment options (Reeves and Piefer, 2006). The increased use of combination ART, has drastically improved the efficacy of ART leading to reductions in both morbidity and mortality, and increasing life expectancy of HIV infected individuals. The use of efficacious ART is life long and the benefits conferred by its usage have turned HIV/AIDS from a death sentence to a chronic and manageable infection (Brenner et al., 2007; Murphy et al., 2001). The treatment options available, have also improved over the years with the development of newer, less toxic, longer-acting and more effective compounds capable
of better suppression of viral replication (Wainberg and Jeang, 2008).

The effectiveness of anti-retroviral therapy in HIV infected individuals is often determined by routine monitoring and evaluation of virologic and immunologic parameters such as HIV viral load and CD4+ T cell counts, respectively. Viral load monitoring involves the quantification of HIV RNA in the plasma, usually; effective ART results in undetectable levels of HIV-RNA in plasma or below 50 copies/ml (AIDS info, 2015). CD4+ T cell counts monitoring is a measure of the number of CD4+ T cells present in the blood of an HIV infected individual. In 2015, WHO adjusted its recommendations for ART initiation, calling for all individuals found to be HIV positive irrespective of CD4+ T cell count to be put on ART (WHO, 2015). Early initiation of ART, limits the extent to which HIV causes destruction of the immune system especially to CD4+ T cell reservoirs and also limits the establishment of latent viral reservoirs which contribute to persistence of viral replication during ART (Jain et al., 2013). This enables infected individuals to live longer, healthier lives while substantially reducing the risk of such individuals transmitting new infections to others (Vernazza et al., 2000). The recommendations also include the provision of ARVs to children under the age of 10 years. Any child aged 10 years or more should be put on ART if their CD4 count is below 500 cells/µl (WHO, 2015).

The use of viral load and CD4+ T cell counts for monitoring ART efficacy among HIV infected individuals has its limitations (Paintsil, 2011). The frequent and reliable use of viral load testing in resource-rich countries complements the quality of care given to HIV infected persons. However, it usage in resource-limited countries is low in frequency or completely absent, and where it is present the sensitivity and reliability is questionable (Katzeinstein, 2003). Viral load quantification requires expensive equipment with high maintenance costs, highly skilled operators, costly reagents and specialized laboratory infrastructure, which are often not present in low-income countries (Katzeinstein, 2003). Despite the recent introduction of relatively cheaper viral load testing techniques like the ExaVir load in some resource-limited countries (Cairns,
2009), the costs of viral load assays remain prohibitive in most low-income countries
(Nkengasong et al, 2009). Viral load testing despite being expensive is a more reliable
predictor of disease progression and treatment failure as compared to CD4+ T cells count
(Hogg et al, 2001). CD4+ T cell counts is more readily available in such settings, even
though it is still costly but less expensive than viral load (Nasi et al, 2015), but lacks the
sensitivity needed for early detection of treatment failure, due to the slower rate of
decline in CD4+ T-cells counts (up to months) following treatment failure (Paintsil,
2011). There are also situations of discordant virology and immunologic responses,
where there is a persistently low or declining CD4+ T cell counts in spite of complete
virologic suppression, or a rising CD4+ T-cell count with increasing or high viral load
(Gazzola et al, 2009). Despite these challenges of viral load monitoring in resource
limited countries, its usefulness in complementing CD4+ cell counts for optimal ART
outcomes is widely accepted (Wang et al, 2010). There is therefore a need to develop
simpler, more robust, low maintenance and cost-effective laboratory techniques to
monitor the clinical efficacy of ARV therapy in these resource-limited countries (Janossy
et al, 2008).

Previously, it has been shown that blood DPPIV levels could inversely correlate to HIV
viral load, and directly correlate with CD4+ T cell levels in adult infected persons
(Ohtsuki et al, 2000). This supported the premise that increased expression of DPPIV
may be immunologically relevant to the course of HIV disease. Additionally, a study
conducted on commercial sex workers (CSWs) from Kenya, found a higher expression
of the enzyme Dipeptidyl peptidase IV in the peripheral blood of HIV exposed
seronegative Female Sex Workers (FSW) (Songok et al, 2010). These findings suggest
that, higher DPPIV levels may have a protective role against HIV acquisition among
female’s commercial sex workers in Kenya.

Dipeptidyl peptidase IV is a 110 kDa protein. The enzyme cleaves N-terminal dipeptides
after proline or alanine residues leading to the release of a number of chemokines that
have this terminal sequence (Ohtsuki et al, 2000). DPPIV is composed of an extra-
cellular domain, a transmembrane region and a cytoplasmic tail. It exists in a dimeric form in different cell types although it also has a soluble isoform (sDPPIV) that is enzymatically active in biological fluids. Soluble DPPIV lacks a transmembrane region and cytoplasmic residue, and can be found in a dimeric form (Havre et al., 2008; Cordero et al., 2009; Iwaki-Egawa et al., 1998). CCL5 or RANTES (Regulated on activation, normal T cell expressed and secreted) a member of Interleukin 8 superfamily of chemokines, competes with HIV for binding to its CCR5 co-receptor, and can be truncated by DPPIV, leading to a 5 fold increase in activity of the truncated form (Boonacker et al., 2003; Herrera et al., 2001; Dong and Morimoto, 1996). The degree of post-translational sialylation of DPPIV is closely related to Tat binding activity of DPPIV, with hypersialylation leading to increased binding of HIV tat protein. This leads to, reductions in DPPIV activity resulting in the loss of DPPIV-mediated T-cell activation (Ohtsuki et al., 2000; Wrenger et al, 1997). HIV typically infects activated CD4+ T-cells and the loss of the DPPIV mediated T-cell activation presumably results in reduced susceptibility to HIV (Ohnuma et al., 2008).

In this study, we determined the relationship between the plasma levels of DPPIV, HIV viral load and CD4+ T cell counts in HIV infected individuals, as a means of evaluating the potential for use of DPPIV levels, as a cheaper and simpler alternative surrogate marker in monitoring HIV disease progression in young individuals receiving ART.

1.2 Problem statement

The use of viral load and CD4+ cell count as markers of ARV drug therapy in HIV+ individuals poses a number of challenges such as poor sensitivity in detecting early treatment failure (Paintstil, 2011). Although viral load testing is reliable, the platform is mostly unavailable for use in resource-limited countries due to a number of disadvantages. Viral load is expensive in terms of technical equipment and maintainance costs, has a complex technology, unaffordable reagents, need for complex and sophisticated laboratory infrastructure. (Katzeinstein et al, 2003). Although cheaper than
viral load, analysis of CD 4+ cell counts is also costly (Nasi et al, 2015), while it also has issues of discordant virology and immunologic responses that pose a challenge in complementing viral load. The use of DPPIV as a potential alternative marker is seen as a potential alternative to addressing the challenges posed by CD4 and viral load testing.

1.3 Justification

DPPIV has been shown to be highly expressed in HIV exposed but sero-negative commercial sex workers hence is a marker of resistance while its levels are also shown to be inversely proportional to the viral load in HIV+ individuals hence its increase may be protective to these individuals and may predict viral load levels and the disease progression. The use of ELISA in its analysis is cheaper and readily available with ease of technique than the RT-PCR required in assaying viral load hence may be employed in resource-limited settings where viral load is not easily accessible. ELISA is also a sensitive and a specific technique hence can help in detecting small changes in DPPIV levels which may assist in early detection in cases of virologic failure. ELISA also portrays concordance, robustness and reproducibility as the assays can be repeated to obtain the same findings.

1.4 Null Hypothesis

There is no difference in the levels of the protein DPPIV in HIV+ individuals before and after six months of ARV drug treatment in comparison to viral load levels and CD 4+ cell counts

1.5 General objective

To investigate the feasibility of using DPPIV protein levels in peripheral blood of HIV+ individuals as a potential surrogate marker in monitoring ARV therapy in comparison to viral load and CD 4+ cell counts
1.6 Specific objectives

1. To determine the demographics of the HIV + individuals recruited in the study by gender and age groups

2. To determine and compare the peripheral blood levels of DPPIV, CD4+ cell counts and HIV viral load measurements among HIV+ individuals recruited in the study before and after starting ART treatment.
CHAPTER TWO

LITERATURE REVIEW

2.1 Cluster of Differentiation 26(CD 26)/ Dipeptidyl Peptidase IV (DPPIV)

CD 26 is widely distributed plasma membrane glycoprotein with intrinsic Dipeptidyl peptidase activity IV (DPPIV) and has a variety of functions in different biological processes within different mammalian tissues (De Meester et al, 1999).

Dipeptidyl peptidase IV (DPPIV) is a member of the large family of proteases (peptidases). DPPIV is also known as adenosine deaminase binding protein (ADBP). It is a serine exopeptidase that catalyses the release of an N-terminal dipeptide provided that the next to last residue is proline, hydroxyproline, dehydroproline or alanine. Only oligopeptides in the trans conformation are able to bind to the active site of DPPIV (Proost et al, 1998).

Initially known as T-cell activation antigen, DPPIV is preferentially expressed on a specific population of T lymphocytes, the CD4+CD45RO+ memory T cells subset, and is upregulated following T cell activation. In addition to being a marker of T cell activation, DPPIV is also associated with the T cell signal transduction processes as a costimulatory molecule (Bonin et al, 1998), and is also involved in a variety of T cell functions such as cell migration and cytokine production (Ohnuma et al, 2011).

2.2 Structure of DPPIV

Human DPPIV is a cell surface glycoprotein, within the serine protease super family, that has a molecular weight of 110 kDa. It is characterized by the Ser-Asp-His triplet in the C terminal region (De Meester et al, 1999) as shown in figure 2.1 below. Cloning and sequencing of the rat DPPIV led to the determination of the primary structure of the enzyme (Ogata et al, 1989). In humans, DPPIV gene is located in chromosome 2 locus 2q24.3 spans approximately 26 kb and contains 26 exons (Thompson et al, 2007). The genomic organization of DPPIV is distinguished from the other serine proteases by
nucleotides encoding the sequences close to the active serine at the 630 residue (Gly-X-Ser-X-Gly) split between two exons (Abbott et al, 1994).

**Figure 2.1:** The structure of CD 26/DPPIV:

The chain of circles shown at the C-terminal catalytic region represents the DPPIV substrates (From Ohnuma et al, 2011). In addition to the amino acids composition of CD 26 structure, this figure also shows the cytoplasmic domain, the membrane domain and the extracellular domain that has the DPPIV (Dipeptidyl peptidase) catalytic region.

Human DPPIV is composed of 766 amino acids. This includes a short cytoplasmic domain consisting of six amino acids, a transmembrane region consisting of twenty four
amino acids and an extracellular domain which consist of dipeptidyl peptidase activity that selectively removes the N-terminal dipeptide from peptides with proline or alanine at the penultimate position (Ohnuma et al, 2011).

The consensus sequence (DW (V/L) YEEE) is located in the N-terminus of the human DPPIV protein with the first two Glu (E) amino acids crucial for enzyme activity (Catalytic site located in this N-terminus containing the catalytic triad shown in the figure 2.1 above while the C terminus containing the alpha/beta hydrolase domain is extremely conserved (Abbott et al, 1999; Abbott et al, 2000). The catalytic site (Ser630-Asp708-His740) is located at a large cavity also known as the central tunnel that is located between the alpha/beta hydrolase domain and the eight bladed Beta propeller domain containing the consensus sequence (DW (V/L) YEE) conserved in S9B protease (Rasmussen et al, 2003).

The use of single amino acid point mutation in the Beta propeller motif led to the identification of Glu205 and Glu206 as essential for the DPPIV enzyme activity (Meintlein et al, 1993), with the central tunnel and the a/b-hydrolase domains both taking part in DPPIV inhibitor binding (David et al, 1993; Hooper et al, 2001). Substrate specificity is controlled by the amino acids lining the opening of the catalytic side pocket (43). His750 single point mutation is important for dimerization (Chien et al, 2004, Kahne et al, 1996). The carbohydrate chains account for around 20% of the molecular weight and cause the observed heterogeneity (Kahne et al, 1996).

2.3 The proteins binding DPPIV and their roles in immunity

Adenosine Deaminase (ADA) interacts with DPPIV. ADA bound to DPPIV has a role in the modulation of the concentration of adenosine at the local extracellular surface that provides negative signals to the T-cell interior by use of adenosine receptors at the cell surface (Callebaut et al, 1998). Moreover, the interaction of ADA with DPPIV provides a co-stimulatory signal in T-cell activation that is mediated by the CD3/T-cell receptor complex but is independent of the ADA enzyme activity (Martin et al, 1995).
DPPIV also binds to the extracellular matrix especially collagen and fibronectin. Several studies have suggested the interaction of DPPIV with the extracellular matrix to the biology of DPPIV-positive tumors (Havre et al, 2008).

DPPIV can also interact with CD 45RO expressed in activated and memory T-lymphocytes that facilitates T-cell activation. CD45RO is a protein Tyr phosphatase linked to a T-cell which normally interacts with DPPIV together with the cytoplasmic domain 2 of CD45 in lipidrafts, which are cholesterol-rich microdomains in cell membranes, which then lead to the modification in cellular signaling events in the peripheral blood T cells (Simmons et al, 2000; Ishii et al, 2001; Torimoto et al, 1991).

DPPIV also interacts with Mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) that binds DPPIV through M6P residues in the carbohydrate moiety of the DPPIV to mediate the internalization of the DPPIV which is an important interaction for the DPPIV-mediated T-cell activation and migration (Ikushima et al, 2000; Jiang et al, 1997).

The DPPIV mannose 6 phosphorylation increases after the activation of T-cell leading to DPPIV internalization hence inhibition of the DPPIV-mediated T-cell activation (Ohnuma et al, 2011).

CXCR4 chemokine receptor also interacts with DPPIV on T and B cells. The binding of CXCR4 stromal cell-derived factor 1 alpha (SDF-1 α) ligand to its receptor leads to the cointernalization of CXCR4 and DPPIV, and this interaction between CXCR4 and DPPIV may act as a means of regulation of the local SDF-1 α activity (Ohnuma et al, 2011).

The cells which express DPPIV with a degree comparable to that of activated T-cells are sensitive to X4 HIV-infection and X4 HIV-1 mediated apoptosis than the cells expressing lower levels of DPPIV (Callebaut et al, 1998; Ohtsuki et al, 1998). However, the over-expression of DPPIV may suppress the entry of X4 HIV-1 (Callebaut et al, 1998).
Due to the exploitation of the chemokine receptors by HIV-1 for entry into the cell, a number of chemokine may provide a level of protection against the HIV-1 infection due to the occupation of the chemokine to its receptor then followed by the internalization of the chemokine receptor complex. HIV 1 infection was found to be impaired in a T-Cell line by DPPIV inhibitors, pointing out a role of DPPIV in control of the chemokine-receptor interaction (Simmons et al, 2000; Jiang et al, 1997).

The cationic HIV transactivator protein Tat binds to DPPIV and inhibits its enzymatic activity but this depends on the sialylation status of DPPIV (Smith et al, 1998).

In patients with rheumatoid arthritis, Plasminogen type 2 (Pg2) glycoforms located on synovial fibroblasts bind to the residues 313-319 of DPPIV, through the sialic acids of their O-glycan. This may then lead to the activation of urinary type plasminogen activator (u-PA) located in the vicinity, leading to intracellular calcium mobilization.

The protein streptokinase (SK) secreted by streptococci, which in association with Pg facilitates the development of focal infection at the site, can bind to DPPIV expressed by rheumatoid synovial fibroblasts (Gonzalez et al, 2008; Gonzalez et al, 1998).

Studies have also shown that interaction of CD26 with caveolin-1 on antigen-loaded monocytes results in the upregulation of CD 86 therefore leading to enhancement of the subsequent interaction of CD86 and CD 28 on T-lymphocytes hence inducing the antigen specific T-cell proliferation and differentiation (Dong et al, 1996). Caveolin 1 is the costimulatory ligand of human DPPIV which binds to the receptor’s central tunnel, DPPIV enzyme pocket, and the dimerization site through CARMA 1 interaction with the cytoplasmic/membrane domains of DPPIV (Ohnuma et al, 2005, Ohnuma et al, 2004).

2.4 Dipeptidyl Peptidase IV Substrates

The DPPIV enzyme activity is important in enhancing cellular responses to external stimuli (Dong et al, 1996), leading to the activation of T-Cells. A study showed that exogenous and recombinant soluble DPPIV enhanced the proliferation of peripheral
blood lymphocytes after stimulation of the cells with the soluble tetanus toxoid antigen (Ohnuma et al, 2001). It was also demonstrated that Jurkat cells transfected with wild-type DPPIV had consistently a greater activation in comparison to the parental DPPIV negative Jurkat cells or the cells transfected with DPPIV mutant (Tanaka et al, 1993).

Substrates of DPPIV include several chemokines and cytokines. For example, the CCL5 ligand; RANTES (Regulated on activation, normal T cell expressed and secreted), is altered by cleavage of DPPIV. Processed RANTES affects important activities in monocyte chemotaxis and HIV-1 infection (Proost et al, 1999).

Examples of other important chemokines that are substrates to DPPIV enzyme include eotaxin (CCL11), interferon-inducible chemokines (CXCL10), macrophage-derived chemokine (MDC; CCL22), and other chemokines involved in the inhibition of the HIV infection (Ohnuma et al, 2011).

CXCL12(SDF-1 α) is an important chemokine that acts as a chemoattractant for haemapoetic stem cells/haemapoetic progenitor cells and this chemokine is truncated by DPPIV in vitro leading to the loss of its ability to induce migration of the haemapoetic stem cells isolated from the mouse bone marrow (Christopherson et al, 2004; Christopherson et al, 2003).

In glucose metabolism, the inhibition of glucagon-like peptide-1 (GLP-1) degradation by the reduction of DPPIV activity is a strategy to improve the incretin action of GLP-1 in vivo and hence regulating the levels of glucose. This has been used in the treatment of impaired glucose tolerance and type 2 diabetes with more molecules being investigated in clinical trials that inhibit DPPIV (Drucker, 2007; Amori, 2007).

### 2.5 Cluster of differentiation26 (CD 26) in other Immune Cells

In addition to it being a marker of T-Cell activation, DPPIV can also act as an activation marker of B cells and other antigen presenting cells (APC). DPPIV antigen is expressed in CD 20+ B-cells while it is not expressed or found only at low levels in monocytes of a
healthy adult. In dendritic cells, DPPIV is expressed at intermediate levels while only a small fraction of peripheral natural killer cells express DPPIV (Ohnuma et al, 2011).

2.6 Cluster of differentiation 26 (CD 26)/DPPIV in the diagnosis of other diseases

A study has shown that DPPIV can be used in the diagnosis of Tuberculosis Pleural Effusion, which is the pathological accumulation of fluid in the pleural cavity that surrounds the lung often caused by Tuberculosis. (Nuria et al, 2014) The normally used diagnostic techniques of determining Adenosine Deaminase (ADA) activity and determination of the concentration of cytokine IFN-gamma in the pleural effusion are not wholly accurate in indicating whether anti-tuberculosis therapy should be resumed or discontinued. (Jantz and Antony, 2008; Trajman et al, 2007; Valdes et al, 2003; Baba et al, 2008). DPPIV enzyme levels were found to be low in tuberculosis pleural effusion patients than in patients with other benign or malignant with similar levels of sDPPIV in all types of patients. (Nuria et al, 2014).

DPPIV has been shown to be a novel molecular marker for differentiated thyroid carcinoma. This is more than 3 proto-oncogenes previously reported to increase mRNA expression in thyroid carcinomas: c-met, c-erbB-2 and EGF-R. This was conducted using immunohistochemical staining and activity staining which clearly showed that DPPIV enzyme activity staining is the most specific assay for differentiated thyroid carcinoma, yet the easiest to perform. Staining intensity of the enzyme activity was relative to the degree of DPPIV mRNA expression. The study concludes by stating that DPPIV activity staining should be added to the usual pathological examinations in order to distinguish differentiated thyroid carcinomas from benign thyroid diseases. (Tetsuji et al, 1995).

Another study, (Cordero et al, 2015) has shown DPPIV activity levels to be decreased both in serum and synovial fluid of rheumatoid arthritis (RA) patients and this decrease is known to be associated to disease activity with RA patients displaying higher percentages of CD4⁺CD26⁺ T cells and DPPIV cell surface density. (Hagihara et al,
1987; Cordero et al, 2009). Since DPPIV has been defined as an activation marker for T cells, the inflammatory process leads to an up-regulation of the DPPIV expression. (Cordero et al, 2009). The study concludes by stating that DPPIV expression levels in T-helper cells can provide clues to identify biomarkers for earlier stages of rheumatoid arthritis. (Cordero et al, 2015).

In chronic inflammatory bowel disease (IBD), DPPIV serum activity has been shown to decrease suggesting a functional compartmentalization of DPPIV, interpreted as adaptive systemic immune responses to a local inflammatory reaction. Soluble DPPIV may be involved in the pathophysiology of IBD and appear to be useful as a new disease activity measure for IBD. (Varljen et al, 2004).

DPPIV has been shown to suppress malignant transformation of melanocytes to melanoma. This is by degrading autocrine growth factors, which are yet unidentified and hence regulate (suppress) the growth of benign melanocytes. This is characterized by loss of tumorigenicity, reappearance of anchorage-dependent growth, restoration of a block in differentiation, and serum dependence. These findings are quite impressive, as they present direct evidence that the loss of surface expression of DPPIV plays a pivotal role in malignant transformation of melanocytes towards melanoma. (Wesley et al, 1999; Satoshi and Chikao, 1999).

2.7 DPPIV in HIV

In HIV infection, DPPIV can be protective in the following manner. First; DPPIV cleaves and leads to the release of the N-terminal dipeptides where a number of chemokines have this terminal sequence. For example, RANTES (Regulated on activation, normal T cell expressed and secreted), which is an Interleukin 8 superfamily chemokine which normally competes with HIV for its CCR5 co-receptor, is truncated through cleavage by DPPIV, hence creating a 5 fold increase in its activity (Ohtsuki et al, 2000; Boonacker et al, 2003; Dong and Morimoto, 1996).
Secondly, the degree of sialylation as a post-translational modification of DPPIV is closely related to binding of Tat and several HIV-related partial peptides (Ohtsuki et al., 2000). Therefore hypersialylation of DPPIV leads to binding of HIV tat protein. Tat reduces the DPPIV activity leading to loss of DPPIV-mediated T-cell activation. The activation of T-cells is a major factor in the disease progression of HIV and loss of the DPPIV mediated T-cell activation leads to protection against HIV (Ohnuma et al., 2008).

DPPIV enzyme activity may confer relative resistance to HIV-1 infection. A study showed that Jurkat transfectants with a comparable phenotype expressing wildtype DPPIV were relatively resistant when compared with either the parental Jurkat cell lacking DPPIV or the transfectants expressing mutant DPPIV. (Morimoto et al., 1994).

A study tried to determine whether the plasma sDPPIV levels have clinical relevance in HIV-1 infected individuals. After measuring the concentration and DPPIV enzyme activity of plasma sDPPIV, there was a decreased DPPIV enzyme activity in HIV-1 infected individuals. (Hosono et al., 1999).

2.8 Viral load monitoring and CD 4+ Cell counts

HIV RNA (viral load) and CD4 T lymphocyte (CD4) cell count are the two surrogate markers of antiretroviral treatment (ART) responses and HIV disease progression that have been used for decades to manage and monitor HIV infection.

Viral load is a marker of response to ART. A patient’s pre-ART viral load level and the magnitude of viral load decline after initiation of ART provide prognostic information about the probability of disease progression. (Murray et al., 1999). The key goal of ART is to achieve and maintain durable viral suppression. Thus, the most important use of the viral load is to monitor the effectiveness of therapy after initiation of ART.

Measurement of CD4 count is particularly useful before initiation of ART. The CD4 cell count provides information on the overall immune function of an HIV-infected patient. The measurement is critical in establishing thresholds for the initiation and
discontinuation of opportunistic infection (OI) prophylaxis and in assessing the urgency to initiate ART. (HIV Surrogate Marker Collaborative Group, 2000).

2.9 Limitations of Viral load monitoring and CD 4+ Cell counts

Viral load monitoring is important and complements the quality of care given to HIV + individuals. However, its use in resource-limited countries is low or non-existent due to expensive equipment with high maintenance costs, highly skilled operators, costly reagents and specialized laboratory infrastructure, which are often not present in low-income countries (Katzeinstein, 2003).

CD4+ T cell counts is more readily available in these resource-limited countries, but lacks the sensitivity needed for the early detection of treatment failure, because of the slower rate of decline in CD4+ T-cells counts, which can take up to months, after failure of treatment (Paintsil, 2011). Situations of discordant virology and immunologic responses- a persistently low or declining CD4+ T cell counts despite complete virologic suppression, or a rising CD4+ T-cell count coupled with increasing or high viral load (Gazzola et al, 2009).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted in a group of young individuals enrolled in the Lea Toto programme, an initiative of the Children of God Relief Institute (COGRI) that provides home based HIV care services to infected children and their families. The collection of samples was done at the various Lea Toto Centres namely: Kangemi, Dagoretti, Kawangware, Dandora, Mukuru, Zimmerman, Kibera and Kariobangi communities of Nairobi, Kenya. The laboratory procedures were later carried out at the Nyumbani Diagnostic Laboratory located at the Nyumbani Children’s Home and the Kenya Medical Research Institute (KEMRI).

Individuals accepted into the Lea Toto programme, undergo a clinical evaluation of their blood for HIV viral load and CD4+ T cell counts, which are routinely monitored to determine when ART can be initiated. The enrolled are also offered routine prophylaxis and treatment for opportunistic infections. The nutritional status is also closely monitored, and parents or caregivers are advised on a balanced diet. During the period of study, the Kenyan ART guideline for young individuals was that all individuals below 10 years old, ART is started automatically regardless of the CD4+ cell counts but for individuals above 10 years old, ART is started once the CD4+ T cell counts go below 500 cells per mm$^3$. The choice of ART was Nucleoside Reverse Transcriptase Inhibitors (NRTIs) combination chosen was either AZT/3TC or ABC/3TC, but TDF/3TC could be used for adolescents; the Non-Nucleoside Reverse Transcriptase Inhibitors (NNTRI) selected depended on exposure to NVP during pregnancy; those exposed were to be put on a Protease inhibitor (PI) namely LPV/r; for those not NVP-exposed, either NVP or EFV was to be used according to the age and/or weight of the child.
3.2 Study design and study population

The study design was a cohort comparative before and after study. The study participants were all HIV positive young individuals receiving care services under the Lea Toto programme at the Lea Toto Centres located in the mentioned low income areas of Nairobi. A total of 76 HIV positive young individuals between the ages of <1 year to 20 years from all the Lea Toto centres in Nairobi had their samples used based on their availability.

3.3 Sample size determination

Convinience sampling method was used where an arbitrary number of 76 participants were recruited into this study. The rationale for using arbitrary number was partly due to the fact that this was a unique group of minors where assent was to be sought from different levels. From the 76 participants, blood was collected before and after ART initiation leading to a total of 152 blood samples.

Sample distribution

The Lea Toto programme has eight centres and the distribution of the young study participants in the centres are such that out of the 76 whose samples were used in this study, 13 were from Kawangware, 10 were from Kibera, 3 were from Mukuru, 4 from Dandora, 14 from Kangemi, 7 from Zimmerman, 3 from Dagoretti while 22 were from Kariobangi. as shown in Table 3.1 below.
Table 3.1: Distribution of study participants in the Lea Toto centres

<table>
<thead>
<tr>
<th>Lea Toto Centre</th>
<th>Kawang ware</th>
<th>Kibe ra</th>
<th>Mukur u</th>
<th>Dandor a</th>
<th>Kangem i</th>
<th>Zimmer man</th>
<th>Dagoretti</th>
<th>Kario bangi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of study participants</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>14</td>
<td>7</td>
<td>3</td>
<td>22</td>
</tr>
</tbody>
</table>

3.4 Sample collection and preparation

A blood sample was collected before ART initiation and second blood sample collected an average of 7.2 months after ART initiation. A total of 152 samples were therefore collected. Five ml of whole blood was collected from the median cubital vein in K3-EDTA vacutainers tubes (BD, Franklin Lakes, New Jersey), and transported within an hour to the laboratory on ice packs. CD4+ T-cell counts determination was done and the remaining blood was processed by centrifuging at 1500 rpm for 7 minutes. The plasma obtained was used for HIV viral load quantification then refrigerated at -80°C for a month and later used for soluble DPPIV quantification.

3.5 DPPIV quantification by ELISA

Soluble DPPIV plasma levels was quantified using Human DPPIV/CD26 Quantikine ELISA kit (R&D Systems, Minneapolis MN) catalogue number DC 260 according to the manufacturer’s recommendations. DPPIV capture antibodies were coated onto flat bottomed 96 well plates and incubated overnight at room temperature. Following the overnight incubation, the plates were washed three times using a wash buffer (included
in kit), and then blocked using 300µl of 1% Bovine Serum Antigen (BSA) reagent
diluent (Life Technologies-Thermo Fisher Scientific, USA) added to each well followed
by a 1 hour incubation. 100µl of samples, standards or controls were then added to the
respective wells in duplicate and incubated for two hours at room temperature. The
standards were added serially by diluting from a top concentration of 2000pg/ml to a
lowest concentration of 15.625pg/ml. The plasma samples were diluted using 10% BSA
reagent in a volume/volume ratio of 1:1000. After the incubation, the plates were washed
thrice using wash buffer, and detection antibody at a concentration of 36 ng/ml, was then
added to each well. The plates were then incubated for two hours at room temperature,
and subsequently washed thrice using the wash buffer, and 100µl of the Streptavidin-
HRP enzyme conjugate was then added to each well followed by twenty minute
incubation at room temperature in the dark. Following the incubation, the plates were
washed thrice, and a substrate made from TMB (3, 3’, 5, 5; - tetramethylbenzidine)
solution in hydrogen peroxide was added to each well, and then incubated for twenty
minutes at room temperature away from direct light. Following this incubation, 50µl of
the stop solution was then added to each well, and plates read immediately at 450 nm
with a correction wavelength of 540 or 570 nm on a MULTISCAN EX ELISA Reader
(Thermo Scientific, Massachusetts, USA). The optical densities (ODs) of standards were
used to generate a standard curve, which was in turn used for the determination of the
sample concentrations. Subsequently, the sample concentrations were then multiplied by
1000 (the plasma dilution factor) and normalized by subtracting the ODs of the negative
controls (reagent diluent only).

3.6 Viral load quantification

HIV-1 RNA or viral load quantification in blood plasma was performed by RT-PCR
using Roche Amplicor HIV-1 Monitor Test kit that is fully automated. In brief, the
standard protocol is as follows: plasma specimens were treated using guanidine
thiocyanate or ultracentrifuged for low-level quantitation. Plasma specimens or HIV-1
quantitation standards (QS) with known RNA copy numbers, were first reverse
transcribed into cDNA using RT-PCR in the presence of excess deoxynucleoside
triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine,
deoxyuridine and thymidine triphosphates, then quantified by RT-PCR. After the reverse
transcription of the HIV-1 and QS target RNA, the reaction mixture was heated to
denature the RNA: cDNA hybrid and expose the HIV-1 and QS target sequences. The
sequences were then amplified to create an amplicon 142 base pairs long in the area of
the HIV-1 genome between the primers SK 431 and SK462. The enzyme used is the
thermostable recombinant enzyme *Thermus thermophilus* DNA polymerase (rTth pol). In
the presence of manganese and under the appropriate buffer conditions, rTth pol has
both reverse transcriptase (RT) and DNA polymerase activity.

After PCR reaction, the HIV-1 and QS amplicons were chemically denatured to form a
single stranded DNA by the addition of a denaturation solution and aliquots added into
separate wells of a micro-plate coated with HIV specific and QS-specific oligonucleotide
probes. Later, the substrate (Horse radish peroxidase-avidin conjugate) was added to the
probes detected by 3, 3’, 5, 5’- tetramethylbenzidine (TMB). An automated micro-well
plate reader was used in reading the optical densities (OD) after the reaction was stopped
by the addition of a weak acid. The amount of HIV-1 RNA in the sample was calculated
using a ratio of the optical densities of the total HIV-1 RNA and the total HIV-1 QS RNA
optical density and the input number of QS RNA copies that was incorporated into each
individual patient sample at known copy numbers during the specimen preparation. The
input number of QS RNA is lot- specific and is entered by the user before the reaction.
In addition to the quantitative standards, three additional controls accompanied the kit,
these were; the negative; lowpositive and high-positive tested in each assay run provided
with the kit. The kit does not specify the exact identity of the controls.

3.7 CD4+ T-cell counts quantification

The quantification of the CD4+ T cell counts was also done using a FACS Calibur
cytometer (BD). 20 µL of BD Tritest CD4/CD8/CD3 reagent was added to each BD
Trucount tube above the stainless steel retainer without disturbing the pellet. 50 µL of well-mixed anticoagulated whole blood was then pipetted into each tube, without smearing the blood onto the sides of the tube, for enhanced accuracy of CD4+ T cell count quantification. Each tube was then capped and mixed gently by vortexing, then incubated for 15 minutes in the dark at room temperature. 450 µL of 1x BD FACS lysing solution was then added to each tube, capped and gently vortexed to mix. The tubes were then incubated for 15 minutes in the dark, and subsequently the cells were suspended and analyzed on the flow cytometer. The flow data was acquired using the BD Multiset software, with the threshold adjusted prior to data acquisition to minimize background noise and to ensure that populations of interest were included in the data acquisition. Data analysis was done using BD Multiset software, which was also used for calculating the absolute number of CD4+ T cell cells/µL per sample.

3.8 Demographics of the study participants

This was done by the collection of gender and age information from the records at the various Lea Toto Centres. They were later presented by use of graphs and tables.

3.9 Data Analysis

Data analysis was performed using the Graphpad Prism version 5.0. Comparison of general DPPIV, viral loads and CD4+ T cell counts before and after initiation of ART, was done using paired t-tests. Correlational analysis of DPPIV levels with viral load and CD4+ T cell counts was conducted using Pearson’s correlation coefficient. Categorized data comparison for males and females before and after ART was done using paired t-tests while the general differences between male and female young individuals was done using Mann-Whitney test statistics. Data categorized into different age groups before and after ART was analyzed using paired t tests while general differences between the age groups were compared using Mann-Whitney test statistics. All p-value below 0.05 was considered to be statistically significant and reported.
3.10 Ethical considerations

Ethical approval for this study was obtained from the Kenya Medical Research Institute Ethics committee with an SSC (Scientific steering Committee) approval number 2344 and permission for use of samples was provided by the Children of God Relief Institute (COGRI) - The umbrella body which governs the Lea Toto programme and the Nyumbani Children’s home. Informed consent was provided by parents, guardians or grown up children capable of giving consent-under the age of 18 but can comprehend and understand the benefits and risks of the research.
CHAPTER FOUR

RESULTS

4.1 General results obtained from all the patients.

The table showing the specific results obtained from all patients for DPPIV, viral loads and their CD 4+ Cell counts together with their ages and sexes is shown in appendix 2. The DPPIV results being in Pg/Ml, the viral loads are in copies/ml while CD 4+ cell counts are in cells/mm$^3$.

4.2 Descriptive statistics/Demographics of study participants

Out of these 76 individuals, 34(44.7%) were male while 42(55.3%) were female with an average age of 7.8 years.

![Pie chart showing the distribution of female study participants in relation to male study participants](image)

Figure 4.1: Pie chart showing the distribution of female study participants in relation to male study participants
Their age distribution were such that, 14 were infants below 2 years of age, 32 were children between ages of 3 to 9 while 30 were young people of ages 10 to 20 as shown in Figure 4.2 below.

Figure 4.2: Histogram showing the age distribution of the study participants by gender in the Lea Toto programme in Nairobi, Kenya.

4.3 DPPIV general results with viral loads and CD 4+ cell counts

The average levels of DPPIV in the blood of study participants (n=76) before initiation of ART was 830800±438000 pg/ml which significantly rose after initiation of ART to 972800±606200 pg/ml (p=0.0017) (Figure 4.3 a). The average viral load in HIV infected study participants declined from 164600 ± 177170 copies/ml before ART initiation to 31960 ± 8497 copies per Ml (p<0.0001) after ART initiation. The average CD4+ Cell counts was 663.9 ± 598.8 cells per mm³ which increased significantly after ART initiation to 963.8 ± 930.2 cells per mm³ (IQR-402) (p<0.0001) (Figure 4.3 b). Next we compared the relationship between increases in DPPIV levels and declines in viral loads following ART initiation in study participants using Pearson's correlational
analysis which had an R of 0.26 ($p = 0.0246$) showing the presence of very weak correlation between DPPIV increase and viral load decrease after ART. (Figure 4.3 c). The correlation between DPPIV increase and the CD4$^+$ cell counts increase using the same Pearson’s correlational coefficient had an R of 0.14 ($p = 0.2236$) showing no correlation between DPPIV increase to CD4$^+$ cell counts increase after ART. (Figure 4.3 d). This showed that DPPIV levels in HIV infected study participants before and after the initiation of ART was inversely proportional to the viral load levels but directly proportional to the CD4+ T cell counts. Figure 4.3 below represents these findings:
c

$R^2 = 0$, $R = 0.26$

$p = 0.024$

$d$

$R^2 = 0$, $R = 0.14$

$p = 0.22$

CD4+ cell counts INCREASE

DPP1V/CD26 INCREASE

VIRAL LOAD DECREASE
Figure 4.3: Comparison of DPPIV against a number of parameters.

**Figure 4.3 a:** showing the comparison between CD 26/DPPIV levels (pg/ml) and viral load levels (copies/ml) before and after ART. The CD 26/DPPIV levels increased significantly (p=0.0017) after ART while the viral load levels decreased significantly (p<0.0001) after ART showing the inverse proportionality relationship between CD 26/DPPIV and viral load levels. **Figure 4.3 b:** showing the comparison between CD 26/DPPIV (log pg/ml) and CD4+ cell counts (log cells/mm3) before and after ART. The CD 26/DPPIV levels increased significantly (p=0.0017) after ART while the CD4+ cell counts also increased significantly (p<0.0001) after ART showing the direct proportionality relationship between CD 26/DPPIV and CD4+ cell counts. **Figure 4.3 c:** showing Pearson’s correlational analysis between the change in CD 26/DPPIV and the change in viral load after ART. This comparison has a weak correlation with r=0.26 and p=0.0214. **Figure 4.3 d:** showing Pearson’s correlational analysis between the change in CD 26/DPPIV and the change in CD 4+ cell counts after ART. This comparison shows no correlation between CD 26/DPPIV and CD4+ cell counts with r=0.14 and p=0.2236.

CD 26/DPPIV-Cluster of Differentiation 26/Dipeptidyl Peptidase IV, Error bars represent standard error of the mean (s.e.m), * represents p < 0.05, ** p <0.01, *** is p <0.001.

**4.4 DPPIV, viral loads and CD 4+ cell counts in male and female study participants**

The levels of DPPIV in male study participants rose significantly from a mean of 851800 ± 485500 pg/ml before ART, to 1015000 ± 663000 pg/ml after ART (p=0.0112), and similarly in female the same rose from 813700 ± 480500 pg/ml before ART to 938700 ± 605500 pg/ml after ART (p=0.0526). When we compared the change in DPPIV levels in male vs female study participants before and after ART using the Mann-Whitney test we found that p=0.7460 which is not statistically significant therefore there is no significant change in DPPIV with gender (**Figure 4.4 a**).

The viral load levels dropped significantly in the male study participants from a mean
range of 163600 ± 205740 copies/ml before ART, to a mean of 35900 ± 10610 copies/ml after ART ($p=0.0031$). In female study participants, the viral load levels declined significantly from a mean range of 165400 ± 140210 copies/ml before ART to a mean range of 27250 ± 8497 copies/ml after ART ($p=0.0007$). There were no difference in HIV viral load between the male and the female study participants before and after ART (Mann-Whitney $p=0.9875$) (Figure 4.4 b).

The CD4+ T cell counts before and after ART in male study participants increased from a mean range of 665.2 ± 694.2 cells/mm$^3$ before ART to a mean range of 952.9 ± 923 cells/mm$^3$ after ART ($p=0.0021$). In female study participants, the CD4+ T cell counts rose significantly from a mean range of 662.9 ± 520.5 cells/mm$^3$ before ART to a mean of 972.7 ± 765.7 cells/mm$^3$ after ART ($p<0.0001$). When the change in CD 4+ cell counts before and after ART in males was compared to the change in CD 4+ cell counts after ART in females by Mann-Whitney test, the $p$ was 0.548 which is statistically insignificant therefore there is no difference in CD 4+ cell counts based on gender (Figure 4.4 c).
Figure 4.4: DPPIV, viral loads and CD 4+ cell counts in male and female study participants

Figure 4.4 a) The average DPPIV levels before and after ART in male and female study participants. The CD 26/DPPIV levels increased significantly after ART in male study participants ($p=0.0112$) but not in female study participants ($p=0.0526$). However, there was no statistically significant difference between male and female participants with a $p$ of 0.746 (Mann-Whitney) b) HIV viral load levels before and after ART in male and female study participants. The viral load levels increased significantly after ART in male study participants ($p=0.0031$) and also in female study participants ($p=0.0007$). However, there was no statistically significant difference between male and female participants with a $p$ of 0.9875 (Mann-Whitney) c) CD4+ cell counts averages before and after the use of ART in male and female study participants. The CD4+ cell count levels increased significantly after ART in male study participants ($p=0.0021$) and also in female study participants ($p<0.0001$). However, there was no statistically significant difference between male and female participants with a $p$ of 0.548 (Mann-Whitney). CD 26/DPPIV-Cluster of Differentiation 26/Dipeptidyl Peptidase IV, Error bars represent standard error of the mean (s.e.m), * represents $p < 0.05$, ** $p <0.01$, *** is $p <0.001$.

4.5 CD 26/DPPIV, viral loads and CD 4+ cell counts in the various age groups

In individuals below 2 years of age, DPPIV levels before (Mean= 784615.4 ± 200000 pg/ml) and after (mean= 998076.9 ± 520000 pg/ml) ART, remained unchanged ($p=0.15$). However, in individuals between ages of 3-9 years, DPPIV levels increased significantly from a mean range of 740800 ± 340000 pg/ml before ART to a mean of 933300 ± 450000 pg/ml after ART ($p=0.0014$). But in study participants between the ages of 10-20, DPPIV levels remained the same even after initiation of ART (mean=949700 ± 705000 pg/ml to 1005000±805000 pg/ml, $p=0.4466$) When we compared the change in DPPIV levels in individuals below 2 years of age to individuals between the ages of 3-9 before ART using Mann-Whitney test, $p<0.0001$ which is statistically significant
indicating that there is a difference in DPPIV between these 2 age groups before ART. When we compared the change in DPPIV after ART between the individuals below 2 years of age to those between ages 3-9, p<0.0001 which is statistically significant indicating a difference in DPPIV between the individuals of below 2 years to those between 3-9 years after ART. When the same was done between individuals below 2 years to young individuals between ages 10-20, p<0.0001 before ART and p<0.0004 after ART which are both statistically significant showing that there is a difference between DPPIV/CD 26 between these two age groups before and after ART respectively. Finally, the change in DPPIV in individuals of ages 3-9 was compared to the change in DPPIV in ages 10-20 before and after ART using Mann-Whitney test giving a p value of 0.2205 before ART and p of 0.581 after ART indicating that there is no statistically significant difference in DPPIV between ages 3-9 and ages 10-20 before and after ART respectively (Figure 4.5 a)

Viral load levels in individuals <2 years remained unchanged, with a mean range of 174246.7 ± 380187 copies/ml before ART and 100604±144840.5 copies/ml after ART (p=0.37). However, in individuals between ages 3-9, viral load levels decreased significantly after ART initiation from a mean range of 218400 ± 245220 copies/ml before ART to a mean of 7364 ±1331 copies/ml after ARVs (p<0.0001). Similarly, the viral load levels in ages 10-20 declined significantly from a mean of 101100 ± 73891 copies/ml before ART to a mean of 27130 ± 23913 copies/ml after initiation of ART (p=0.04) When we compared the change in viral load levels before ART between the individuals of ages 2 and below to those of ages 3-9 using Mann-Whitney statistics, p<0.0001 which is statistically significant indicating that there is a difference in viral load levels between individuals below 2 years of age and those between ages 3-9 before or without ART. When we compared the change in viral load levels after ART between the individuals of ages 2 and below to those of ages 3-9 using Mann-Whitney statistics, p=0.0006 which is statistically significant indicating that there is a difference in viral load levels between individuals below 2 years of age and those between ages 3-9 after or
with ART. When the change in viral load levels before and after ART was compared between individuals below 2 years of age to those study participants between ages 10-20 using Mann-Whitney test respectively, p was 0.0003 before ART and p=0.001 after ART which are both statistically significant indicating that there is a significant change in viral load levels between individuals of below 2 years and those between 10-20 before and after ART respectively. When the change in viral load levels before and after ART was compared respectively between the individuals of ages 3-9 to those study participants between ages of 10-20 using Mann-Whitney test, p was 0.0002 before ART and p=0.4303 after ART indicating that there is a significant difference in viral load levels between individuals of ages 3-9 to those between 10-20 before or without ART but there is no significant difference in viral load levels between these two age groups after ART. (Figure 4.5 b)

The CD4+ T cell counts remained unchanged in individuals below 2 years of age with a mean range of 1467.85 ± 1531 cells/ mm$^3$ before ART to 1887.46±1141 cells/mm$^3$ after ART ($p=0.052$). In ages 3-9 the CD4+ T Cell counts increased significantly from a mean range of 472.7 ± 364 cells/mm$^3$ before ART to a mean range of 985.9 ± 570 cells/mm$^3$ after ART ($p<0001$). In study participants between ages of 10-20, the CD4+ T cell counts also increased from a mean of 399.7 ± 240 cells/mm$^3$ before ART to a mean of 529.9 ± 418 cells/mm$^3$ after ART ($p=0.0128$).When the change in CD 4+ cell counts before ART was compared in individuals below 2 years to individuals of ages 3-9 using Mann-Whitney test, p was 0.0058 which is statistically significant indicating that there is a difference in CD 4+ cell counts between these two age groups before or without ART. When the change in CD 4+ cell counts after ART was compared in individuals below 2 years to children of ages 3-9 using Mann-Whitney test, p was 0.0112 which is statistically significant indicating that there is a difference in CD 4+ cell counts between these two age groups after or with ART. When the change in CD 4+ cell counts before and after ART was compared respectively between ages of 2 and below to study participants of ages 10-20 years using Mann-Whitney test, p was 0.0509 before or
without ART and \( p=0.1458 \) after or with ART showing that there is a statistically insignificant difference in CD 4+ cell counts between study participants of ages 2 and below to the ones of ages 10 and 20 before or without ART and after or with ART respectively. Finally, the change in CD 4+ cell counts before and after ART was compared respectively between ages 3-9 and ages 10-20 using Mann-Whitney test giving a \( p \) value of 0.0778 before ART and a \( p<0.0001 \) after ART which shows that there is a statistically insignificant difference in CD 4+ cell counts between ages 3-9 to ages 10-20 before ART compared to a statistically significant difference in the same age groups after ART (Figure 4.5 c).

The levels of DPPIV expressed increased between the ages <1-20 years, plateauing at between 10-20 years.
a

DPPIV/CD 26

Before ART

After ART

***

ns

***

***

<2  3-9  10-20

Ages (years)

b

VIRAL LOAD

Viral Load (copies/ml)

***

ns

<2  3-9  10-20

Ages (years)

c

CD 4 + CELL COUNTS

CD 4 + Cell Counts (cells/mm³)

ns

***

<2  3-9  10-20

Ages (years)
Figure 4.5: DPPIV, viral loads and CD 4+ cell counts in various age groups

**Figure 4.5 a)** DPPIV levels before and after ART in HIV infected young individuals between <1-20 years, before and after anti-retroviral therapy (ART). Comparison in DPPIV in ages <2 years before and after ART was statistically insignificant (p=0.15), in ages 3-9 DPPIV before and after ART was statistically significant (p=0.0014) while in ages 10-20 DPPIV was statistically insignificant after ART (p=0.4466). The figure also shows the level of significance in DPPIV when each age group is compared before ART then after ART.

**b)** The average viral load levels before and after ART in HIV positive study participants between <1-20 years. Comparison in viral load levels in ages <2 years before and after ART was statistically insignificant (p=0.37), in ages 3-9 viral load levels before and after ART was statistically significant (p<0.0001) while in ages 10-20 viral load was statistically significant after ART (p=0.04). The figure also shows the level of significance in viral load levels when each age group is compared before ART then after ART.

**c)** The average CD4+ cell counts before and after ART in individuals aged between <1-20 years. Comparison in CD4+ cell count levels in ages <2 years before and after ART was statistically insignificant (p=0.052), in ages 3-9 CD4+ cell count levels before and after ART was statistically significant (p<0.0001) while in ages 10-20 CD4+ cell count levels were statistically significant after ART (p=0.0128). The figure also shows the level of significance in CD4+ cell count levels when each age group is compared before ART then after ART. CD 26/DPPIV-Cluster of Differentiation 26/Dipeptidyl Peptidase IV, Error bars represent standard error of the mean (s.e.m), * represents p < 0.05, ** p <0.01, *** is p <0.001.
Table 4.1: The average DPPIV, viral load and CD 4+ Cell counts in study participants aged 10-20, children of ages 3-9 and in individuals aged <2 years.

<table>
<thead>
<tr>
<th>Study participants of ages 10-20 years</th>
<th>DPPIV before ART in pg/ml</th>
<th>DPPIV after ART in pg/ml</th>
<th>viral load before ART in copies per ml</th>
<th>Viral load after ART in copies per ml</th>
<th>CD4+ cell count before ART in cells per mm$^3$</th>
<th>CD4+ cell counts after ART in cells per mm$^3$</th>
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<th>Study participants of 3-9 years</th>
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<th>DPPIV after ART in pg/ml</th>
<th>viral load before ART in copies per ml</th>
<th>Viral load after ART in copies per ml</th>
<th>CD4+ cell count before ART in cells per mm$^3$</th>
<th>CD4+ cell counts after ART in cells per mm$^3$</th>
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<th>Study participants below 2 years of age</th>
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<th>DPPIV after ART in pg/ml</th>
<th>viral load before ART in copies per ml</th>
<th>Viral load after ART in copies per ml</th>
<th>CD4+ cell count before ART in cells per mm$^3$</th>
<th>CD4+ cell counts after ART in cells per mm$^3$</th>
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<td>100604</td>
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Table 4.1 showing the average DPPIV, viral load levels and CD4+ Cell counts in the various age specific groups before and after ARV use. This shows the general mean increases in DPPIV before and after ARVs across the age-groups, the reduction in mean levels of the viral load copy numbers and the increase in CD 4+ cell counts before and after the use of ARVs across the different age groups.
CHAPTER FIVE  
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

DPPIV is an ectoenzyme that cleaves penultimate proline or alanine residues from the N-terminal of dipeptides or polypeptides. DPPIV exists either in a membrane bound or soluble isoform, the latter circulates in the peripheral blood (Ohnuma et al., 2008). DPPIV has been associated with costimulatory functions in T-cells associated with its ability to bind to adenosine deaminase or CD45 leading to the T-cell activation (Ohtsuki et al., 2000; Boonacker et al., 2003; Herrera et al., 2001). Previously, it was observed that there was a higher expression of DPPIV in the blood of female sex workers who are highly exposed but persistently are HIV sero-negative (HESN) (Songok et al., 2010). Based on these earlier observations, in this study we sought to evaluate if plasma levels of DPPIV in HIV infected young individuals, could be used a surrogate marker for disease progression.

The present study, demonstrated for the first time that the levels of DPPIV in HIV infected young individuals, was inversely proportional to HIV viral load but directly proportional to CD4+ T cells counts. In multiple instances, DPPIV levels, HIV viral loads or CD4+ T cell counts were dependent on ART but independent of gender. The levels of DPPIV expressed tended to increase between the ages <1-20 years, plateauing at between 10-20 years. Anti-retroviral therapy caused significant declines in DPPIV levels in males but not females. Often, the levels of DPPIV remained unaffected by ART. The exception was in young individuals aged 3-9 years, who had higher levels of DPPIV following ART initiation. Based on the same age stratification, the HIV viral load levels were highest in individuals between 3-9 years of age prior to ART compared to those below 2 years or those between 10-20 years of age. ART effectively reduced the HIV viral load in individuals between 3-9 years and those between 10-20 years, but failed to do the same in individuals below 2 years of age. Similarly, ART improved the CD4+ T
cell counts in ages 3-9 years and those between 10-20 years, but failed to do so in individuals below 2 years.

The key finding of this study was that DPPIV levels corresponded to those of CD4+ T cell counts and inversely to HIV viral load, suggesting that DPPIV has the potential for use as a marker to predict HIV disease progression. The immunological importance is not yet clear at this point. However, evidence from studies conducted by others suggest that dysregulation of the immune system heightens the expression of DPPIV. For instance, its levels are elevated in the serum of individuals with metastatic colorectal carcinoma and malignancy in papillary carcinoma of the thyroid gland compared to healthy subjects (de la Haba Rodriguez et al, 2002; Aso et al, 2012). Elevated DPPIV levels in serum of individuals with type 2 diabetes produced poor responses to sitagliptin treatment (Bunupuradah et al, 2006). This finding also corresponds with results from a cohort study showing that Highly Exposed but Uninfected commercial sex workers in Nairobi had highly elevated DPPIV levels, with these women having low levels of the virus showing that DPPIV could have some protective properties against the virus as depicted by these sex workers who do not seroconvert which is consistent with Songok et al, 2010. Our findings also suggest that the administration of ART to HIV-treatment naïve patients boosted the levels of DPPIV after ART. In a study conducted in patients with primary biliary cirrhosis, levels of DPPIV positive peripheral blood lymphocytes were boosted to the range observed in healthy subjects in addition to improving the liver function parameters of the subjects after the administration of ursodeoxycholic acid treatment (Adler et al, 1993).

An outstanding observation from this study was the poor response to ART in children below 2 years of age, based on changes in CD4+ T cell counts, decline in HIV viral loads and likewise DPPIV. This could be attributed to toxicity of most ARVs and the limited dosing alternatives; where very few are available in suspension form. This often forces caregivers to decrease the duration of the administration of the drugs or reduce dosages administered (Natella and Phelps, 2012). Despite the presence of liquid
formulations, challenges still exist in the administered form of the drugs with most still existing in tablet form hence posing a challenge in swallowing of the drugs in this age group (Bunupuradah et al., 2006; Heald et al., 1998). The immune systems of children in this age group are still developing therefore majority may not demonstrate immunological confidence and viral control with the organ systems still immature posing a challenge in absorption and metabolism of the drugs (Fukushima et al., 2009; Neely and Rakhmanina, 2011).

The general viral load levels and CD 4+ cell counts observed in this study before and after ART is consistent with expected outcomes from other studies with use of these drugs (Carter, 2016). In clinical trials studies, the decline in viral load levels after initiation of ART led to reduced risk of progression to AIDS or death (Murray et al., 1999; Marschner et al., 1998; Thiebaut et al., 2000). For patients under ART, the CD 4+ cell counts should increase to a steady level which is used to assess the patients immunologic response. This explains the increase in CD 4+ cell counts in this study after introduction of ART (Kaufman et al., 2003). The same observation in viral load and CD 4+ cell counts in the children between 3 to 9 years of age and those between ages 10-20 in this study is consistent with these other studies (Murray et al., 1999; Marschner et al., 1998; Thiebaut et al., 2000; Kaufman et al., 2003).

5.2 Conclusion

1. This study showed the demographics in the study population depending on age and gender.

2. This study also provided a strong rationale of the potential for use of DPPIV as marker for ART treatment efficacy in young individuals. This is based on the observation that DPPIV levels directly corresponded with CD4+ T cell counts but inversely corresponded with HIV loads in these young study participants with ART use. Therefore, demonstrating the potential for use of DPPIV in developing diagnostic kits for ART monitoring and probably be used in future in the development of vaccine initiatives
against the virus.

Therefore we disregard the null hypothesis and adopt the alternate hypothesis showing the presence of a difference in the levels of DPPIV in comparison to the viral loads and CD 4 + cell counts.

5.3 Recommendations

This work shows that DPPIV can be used as an alternative marker to monitor ART in HIV + individuals. This may lead to the construction of a simple antibody-antigen kit borrowing the ELISA principle to analyze DPPIV in plasma as a surrogate marker for the monitoring of HIV infected individuals.

5.4 Study Limitations

The major study limitation was that it was not feasible within the time duration to obtain more samples. Therefore only 76 samples were obtained calling for a need to upscale this study with an increased number of samples. In addition, the time to get the ethical approval was long and there were longer periods in procurement of study materials.
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APPENDICES

APPENDIX 1: CONSENT FORMS, ASSENT DOCUMENTS AND PARENTAL PERMISSION FORMS IN ENGLISH AND KISWAHILI

Consent form

English consent form

Version number 2.0 Effective date: 12/03/2013

Information sheet for participants in the research proposal titled: The feasibility of using dipeptidyl peptidase IV/CD 26 as a surrogate marker in monitoring anti-retroviral drug therapy among a group of HIV+ individuals in Nairobi, Kenya.

Name of principal investigator.............................Ayub Kiprotich Maina

Name of organization…………………………………JKUAT/KEMRI

Study location: Nyumbani diagnostic laboratory, Nairobi.

Purpose of the study: We are asking you to participate in a research study. This is a study that aims at investigating the possibility of using a protein called CD 26/DPPIV to predict the levels of the virus in HIV+ individuals. This is a new test that is cheaper and can improve the monitoring of HIV patients before and during the use of ARVs.

Description of the research

The eligible participants should be youth who are HIV+ before and six months after initiation of ARV treatment. The patient is required to give 3 ml of blood sample after consent. The first sample will be collected immediately then another one after six months from the start of ARVs. The blood samples will be collected in vacutainer tubes with a suitable anti-coagulant. They will then be stored in cool boxes with ice to preserve them and then shipped to the laboratory for analysis. While in the laboratory, they will be stored in a refrigerator as the analysis of the blood continues.
Benefits to participants

The participant will get a free test on viral load, CD4+ cell count, CD 26/DPP IV levels and the results will be made available to the patients. The generated results will benefit other HIV patients in future on establishing the usefulness of the CD 26/DPPIV test.

Risks and discomforts of being in the study

The study will collect personal information from you like your name, your HIV status, the use of ARVs, if you have suffered any other disease in the near past or if you are tolerant or allergic to any drug. This information will be kept private. We will not keep your name with your sample or give your sample a code number that could identify you. Therefore, nobody at the repository or who studies your sample will know it came from you. All these information will be kept safely under lock and key or password protected computer files so that no unauthorized persons can access the information.

We will collect 3 ml of blood samples using a needle and a syringe/vacutainer tubes. This will be done immediately and after you come back after six months. The puncture of the needle may be uncomfortable and leave a bruise; the drawing of blood may cause pain where the needle enters the vein. Very rarely, some people experience light-headedness or possibly faint during this procedure. This will be reduced by allowing you to take plenty of rest immediately after the procedure. There is also a small chance of infection occurring at the site where we take your blood. This will be avoided by wiping the skin region with antiseptic like methylated spirit before drawing blood and covering the region with cotton wool soaked in the same antiseptic for some time after drawing the blood.

Confidentiality/Privacy of information

We will protect your privacy in several ways. While KEMRI will keep your signed consent form, only the KEMRI ERC and JKUAT ethical review may request to see it.
Your name will be kept differently from your sample while any code number given to the sample will be in such a way that will not easily identify you therefore nobody working with your sample will know it came from you.

At the end of the study, we plan to write a report. The report will not bear any information regarding your personality, for example, your name identity. We assure you of confidentiality of such information. Thus, we also need your permission to use the results for writing a report.

**Right to refuse or withdrawal**

You do not have to take part in this research if you do not wish to do so and this will not affect your ability to receive treatment at the hospital. You may stop participating at any time that you wish to without losing any of your right as a patient. Incase you agree to participate in the study; we will ask you to read and sign the consent form attached to this sheet. You will be given a copy of the signed and dated consent form to keep.

In case of any questions or concerns regarding this research, please contact the principal investigator by:

Ayub Kiprotich Maina

P.O Box 13096-00100,

Nairobi, Kenya.

0733937340

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact anonymously if you wish-

The secretary,
KEMRI Ethics Review Committee,

P.O Box 54840-00200,

Nairobi, Kenya.

Tel: 020-2722541,

0722205901,

0733400003

Email address: erc@kemri.org.

Consent form/Certificate of consent

I the undersigned will like to confirm that as I give consent to participate in the study, it is with a clear understanding of the objective and condition of the study and with the recognition of my right to resign from the study.

I……………………………………., do hereby give consent to Dr/Mr ….........................................to include me in the proposed research and test. I have been given the necessary information to understand that there might be some risks involved in drawing of blood. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefit due to me. The proposal has been explained to me in the language I am conversant in.

Name of patient…………………………………………………………

Patient’s signature……………………………………………………

Date……………………………………………… Place…………………………
Kiswahili consent form

Nambari la Toleo: 2 Tarehe: 12/03/2013

Maelezo kwa watakaohusika katika utafiti lenye mada ifuatayo: Uchunguzi wa CD 26/Dipeptidyl peptidase kama unawezaashiria utumizi wa madawa ya ARVs kwa vijana walio na virusi vya HIV.

Jina la mtafiti mkuu..........................Ayub Kiprotich Maina

Jina la shirika........................................JUAT/KEMRI

Jina la kituo cha utafiti: Nyumbani diagnostic laboratory, Nairobi.

Lengo la utafiti: Tunaomba kushiriki kwako katika utafiti huu. Utafiti huu unatarajia kuchunguza uwezekano wa kutumia kipengele cha mwili CD 26/DPPIV kutabiri kwang性别 cha virusi kwa wanaougua HIV. Hii ni njia mpya ya bei nafuu na itasaidia katika uchunguzi wa wanaougua virusi vya ukimwi kabla na baada ya kutumia madawa ya ARVs.

Maelezo ya utafiti

**Faida ya utafiti**

Watakaoshiriki wataweza kupimwa viwango vya viral load, CD4+ cells na CD 26/DPPIV na matokeo yote yatafunuliwa kwa wakati wa utumizi wa kipengele hicho cha CD 26/DPPIV.

**Hatari ya utafiti**

kwa kutumia antiseptiki inayofaa kama vile spirit ya hospitali. Hii ni kabla na baada ya kutoa damu kwa eneo hilo.

Siri ya maelezo

Tutalinda maelezo tutakayopokea kutoka kwako kwa siri.Tutumia njia zifuatazo. KEMRI italinda fomu hii ya ruhusa kutoka na mtu mwingine yeyote hawezi kutazama isipokuwa tume la KEMRI ERC na JCUAT ERC. Hatuwezi kamwe kuweka sampuli ya damu yako ikiwa na jina lako ama tuipatie nambari ambayo mtu yeyote atakutambua. Kwa hivyo hakuna yeyote atakayejua ilitoka kwako.

Wakati utafiti huu utafika mwisho, tunapanga kuandika ripoti kamili kuhusiana na matokeo yetu. Ripoti hii kamwe haitakuwa na maelezo yoyote kuhusiana na maneno yanayofaa kuwa siri kwako, kwa mfano, jina lako na chochote kile kinaweza kukutambulisha. Tunakuhakikisha kwamba maelezo kama hayo tayaweka kwa siri. Kwa hivyo tunakuhakikisha kwamba maelezo kamilefu itakyosaidia walio na virusi vya HIV.

Haki ya kukataa na kujiondoa kutoka kwa utafiti

Sio lazima ushiriki katika utafiti huu ikiwa hauna nia ya kufanya hivyo, na chaguo lako kamwe halitakuza kupata matibabu katika hospitali. Unawezatoa kushiriki kwako kwa tukutambulisha haki yako kama mgo mwingine. Ikiwa utakubali kushiriki kwa utafiti huu tafadhali kusema kwa chochote kufanya. Kwa hivyo tunanishia kwamba maelezo kamilefu itakyosaidia walio na virusi vya HIV.

Ikiwa una swali au tatizo lolote kuhusiana na utafiti huu,unaweza kuwasiliana na mtafiti mkuu kwa:

Ayub Kiprotich Maina

P.O Box 13096-00100,
Nairobi, Kenya.
0733937340

Ikiwa una tashwishi yeyote kuhusiana na haki zako kama mshirika wa utafiti huu au hajafurahia kitu chochote katika utafiti huu unawezawasiliana-ukitaka kwa siri:

Karani,

KEMRI Ethics Review Committee,

P.O Box 54840-00200,

Nairobi, Kenya.

Tel: 020-2722541,

0722205901,

0733400003

Barua pepe: erc@kemri.org.

Sahihi ya Ruhusa

Ningependa kuhakikisha ya kwamba natoa ruhusa yangu kushiriki katika utafiti huu kwa sababu nimeelewa malengo na madhumuni ya utafiti huu na kwamba nina haki ya kujiondoa wakati wowote.

Mimi...............................................natoa ruhusa yangu kwa Dr/Mr...........................................
anijumulishe katika utafiti unaopendekezwa. Nimepewa maelezo yote ninayohitaji na kwamba kunawezakua na hatari wakati wa kutoa damu. Nimemhakikishiwa ya kwamba ninawezaaondoa ruhusa yangu bila kutuhumiwa au kupoteza faida yeyote ninayopokea kwa sasa. Yote yanayohusiana na utafiti huu umeelez ewa kwangu kwa lugha ninayoelewa.

Jina la mshiriki....................... Sahihi............................
Tarehe.................................

Jina la mchunguzi......................
Sahihi.................................
Tarehe.................................

Assent documents

English assent document

Version number 2.0    Effective date: 12/03/2013

Information sheet for participants in the research proposal titled: The feasibility of using dipeptidyl peptidase IV/CD 26 as a surrogate marker in monitoring anti-retroviral drug therapy among a group of HIV+ individuals in Nairobi, Kenya.

Name of principal investigator...............................Ayub Kiprotich Maina

Name of organization........................................JLUAT/KEMRI

Study location: Nyumbani diagnostic laboratory, Nairobi.

Purpose of the study: We are asking you to participate in a research study. This is a
study that aims at investigating the possibility of using a protein called CD 26/DPPIV to predict the levels of the virus in HIV+ individuals. This is a new test that is cheaper and can improve the monitoring of HIV patients before and during the use of ARVs.

**Description of the research**

The eligible participants should be youth who are HIV+ before and six months after initiation of ARV treatment. The patient is required to give 3 ml of blood sample after consent. The first sample will be collected immediately then another one after six months from the start of ARVs. The blood samples will be collected in vacutainer tubes with a suitable anti-coagulant. They will then be stored in cool boxes with ice to preserve them and then shipped to the laboratory for analysis. While in the laboratory, they will be stored in a refrigerator as the analysis of the blood continues.

**Benefits to participants**

The participant will get a free test on viral load, CD4+ cell count, CD 26/DPP IV levels and the results will be made available to the patients. The generated results will benefit other HIV patients in future on establishing the usefulness of the CD 26/DPPIV test.

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The study will collect personal information from you like your name, your HIV status, the use of ARVs, if you have suffered any other disease in the near past or if you are tolerant or allergic to any drug. This information will be kept private. We will not keep your name with your sample or give your sample a code number that could identify you. Therefore, nobody at the repository or who studies your sample will know it came from you. All these information will be kept safely under lock and key or password protected computer files so that no unauthorized persons can access the information.

We will collect 3 ml of blood samples using a needle and a syringe/vacutainer tubes. This will be done immediately and after you come back after six months. The puncture of the needle may be uncomfortable and leave a bruise; the drawing of blood may cause
pain where the needle enters the vein. Very rarely, some people experience light-headedness or possibly faint during this procedure. This will be reduced by allowing you to take plenty of rest immediately after the procedure. There is also a small chance of infection occurring at the site where we take your blood. This will be avoided by wiping the skin region with antiseptic like methylated spirit before drawing blood and covering the region with cotton wool soaked in the same antiseptic for some time after drawing the blood.

Confidentiality/Privacy of information

We will protect your privacy in several ways. While KEMRI will keep your signed assent form, only the KEMRI ERC and JKUAT ethical review may request to see it. Your name will be kept differently from your sample while any code number given to the sample will be in such a way that will not easily identify you therefore nobody working with your sample will know it came from you.

At the end of the study, we plan to write a report. The report will not bear any information regarding your personality, for example, your name identity. We assure you of confidentiality of such information. Thus, we also need your permission to use the results for writing a report.

Right to refuse or withdrawal

You do not have to take part in this research if you do not wish to do so and this will not affect your ability to receive treatment at the hospital. You may stop participating at any time that you wish to without loosing any of your right as a patient. Incase you agree to participate in the study; we will ask you to read and sign the assent document attached to this sheet. You will be given a copy of the signed and dated assent document to keep.

In case of any questions or concerns regarding this research, please contact the principal investigator by:

Ayub Kiprotich Maina
P.O Box 13096-00100,
Nairobi, Kenya.
0733937340

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact-anonymously if you wish-
The secretary,
KEMRI Ethics Review Committee,
P.O Box 54840-00200,
Nairobi, Kenya.
Tel: 020-2722541,
0722205901,
0733400003
Email address: erc@kemri.org.

Consent form/Certificate of consent

I the undersigned will like to confirm that as I give assent to participate in the study, it is with a clear understanding of the objective and condition of the study and with the recognition of my right to resign from the study.

I……………………………………., do hereby give consent to Dr/Mr ….........................................to include me in the proposed research and test. I have been given the necessary information to understand that there might be some risks involved in drawing of blood. I have also been assured that I can withdraw my assent at any time
without penalty or loss of benefit due to me. The proposal has been explained to me in the language I am conversant in.

Name of patient………………………………………………………….

Patient’s signature………………………………………………………

Date……………………………………………….   Place……………………

Name of Principal investigator………………………………………

Signature of principal investigator…………………………………

Date…………………………………….   Place…………………………

Kiswahili assent document

Nambari la Toleo: 2   Tarehe: 12/03/2013

Maelezo kwa watakaohusika katika utafiti lenye mada ifuatayo: Uchunguzi wa CD 26/Dipeptidyl peptidase kama unawezaashiria utumizi wa madawa ya ARVs kwa vijana walio na virusi vya HIV.

Jina la mtafiti mkuu………………………………Ayub Kiprotich Maina
Jina la shirika..................................................JKUAT/KEMRI

Jina la kituo cha utafiti: Nyumbani diagnostic laboratory, Nairobi.

Lengo la utafiti: Tunaomba kushiriki kwako katika utafiti huu. Utafiti huu unatarajia kuchunguza uwezekano wa kutumia kipengele cha mwili CD 26/DPPIV kutabiri kwango cha virusi kwa wanaougua HIV. Hii ni njia mpya ya bei nafuu na itasaidia katika uchunguzi wa wanaougua virusi vya ukimwi kabla na baada ya kutumia madawa ya ARVs.

Maelezo ya utafiti


Faida ya utafiti

Watakaoshiriki wataweza kupimwa viwango vya viral load, CD4+ cells na CD 26/DPPIV na matokeo yote yatafunuliwa kwao. Matokeo haya yatasaidia wanaougua ukimwi kwa sasa na kwa siku za usoni kwa kufafanua utumizi wa kipengele hichi cha CD 26/DPPIV.

Hatari ya utafiti

Utafiti huu utahitaji maneno yanayowezakuwa ya faragha kwako kama vile jina lako, hali ya HIV, utumizi wa madawa ya ARVs, ikiwa umeugua ugonjwa yoyote hivi karibuni au ikiwa mwili wako haielewani na madawa fulani fulani. Maelezo haya yatawekwa kwa

**Siri ya maelezo**

Tutalinda maelezo tutakayopokea kutoka kwako kwa siri. Tutatumia njia zifuatazo. KEMRI italinda fomu hii ya ruhusa kutoka kwako na mtu mwingine yeyote hawezi kutazama isipokuwa tume la KEMRI ERC na JKUAT ERC. Hatuwezi kamwe kuweka sampuli ya damu yako ikiwa na jina lako ama tuipati nambari ambayo mtu yeyote atakutambua. Kwa hivyo hakuna yeyote atakayejua ilitoka kwako.

Wakati utafiti huu utafika mwisho, tunapanga kuandika ripoti kamili kuhusiana na matokeo yetu. Ripoti hii kamwe haitakuwa na maelezo yoyote kuhusiana na maneno yanayofaa kuwa siri kwako, kwa mfano, jina lako na chochote kile kinaweza kukutambulisha. Tunakuhakikishia kwamba maelezo kama hayo tayaweka kwa siri. Kwa hivyo tunaomba ruhusa yako kutumia maetokeo tutakayoyapata kuandika ripoti kamilifu itakayosaidia walio na virusi vya HIV.

**Haki ya kukataa na kujiondoa kutoka kwa utafiti**

Sio lazima ushiriki katika utafiti huu ikiwa hauna nia ya kufanya hivyo, na chaguo lako
kamwe halitakuzuia kupata matibabu katika hospitali. Unawezatoa kushiriki kwako katika utafiti huu bila kupoteza haki yako kama mgonjwa. Ikiwa utakubali kushiriki kwa utafiti huu, tunakuomba usome na uweke sahihi kwenye fomu la kuomba ruhusa kutoka kwako. Utapewa fomu hilo lililowekwa sahihi pamoja na tarehe yake ili uitunze.

Ikiwa una swali au tatizo lolote kuhusiana na utafiti huu, unaweza kuwasiliana na mtafiti mkuu kwa:

Ayub Kiprotich Maina

P.O Box 13096-00100,

Nairobi, Kenya.

0733937340

Ikiwa una tashwishi yeyote kuhusiana na haki zako kama mshirika wa utafiti huu au hajafurahia kitu chochote katika utafiti huu unaweza wasiliana-ukitaka kwa siri:

Karani,

KEMRI Ethics Review Committee,

P.O Box 54840-00200,

Nairobi, Kenya.

Tel: 020-2722541,

0722205901,

0733400003

Barua pepe: erc@kemri.org.
Sahihi ya Ruhusa

Ningependa kuhakikisha ya kwamba natoa ruhusa yangu kushiriki katika utafiti huu kwa sababu nimeelewa malengo na madhumuni ya utafiti huu na kwamba nina haki ya kujiondoa wakati wowote.

Mimi...............................................natoa ruhusa yangu kwa Dr/Mr.................................................... anijumulishe katika utafiti unaopendekezwa. Nimepewa maelezo yote ninayohitaji na kwamba kunawezakuwa na hatari wakati wa kutoa damu. Nimehakikishiwa ya kwamba ninawezaandoa ruhusa yangu bila kutuhumiwa au kupoteza faida yeyote ninayopokea kwa sasa. Yote yanayohusiana na utafiti huu umaelezewe kwangu kwa lugha ninayoelewa.

Jina la mshiriki ..................... Sahihi..........................
Tarehe.................................

Jina .................................. la mchunguzi..........................
Sahihi.................................

Parental/Guardian permission form

English parental/guardian permission form

Version number 2.0 Effective date: 12/03/2013

Information sheet for participants in the research proposal titled: The feasibility of using dipeptidyl peptidase IV/CD 26 as a surrogate marker in monitoring anti-retroviral drug therapy among a group of HIV+ individuals in Nairobi, Kenya.

Name of principal investigator............................Ayub Kiprotich Maina
Name of organization: ........................................JKUAT/KEMRI

Study location: Nyumbani diagnostic laboratory, Nairobi.

Purpose of the study: We are asking your child to participate in a research study. This is a study that aims at investigating the possibility of using a protein called CD 26/DPPIV to predict the levels of the virus in HIV+ individuals. This is a new test that is cheaper and can improve the monitoring of HIV patients before and during the use of ARVs.

Description of the research

The eligible participants should be youth who are HIV+ before and six months after initiation of ARV treatment. The patient is required to give 3 ml of blood sample after consent. The first sample will be collected immediately then another one after six months from the start of ARVs. The blood samples will be collected in vacutainer tubes with a suitable anti-coagulant. They will then be stored in cool boxes with ice to preserve them and then shipped to the laboratory for analysis. While in the laboratory, they will be stored in a refrigerator as the analysis of the blood continues.

Benefits to participants

The participant will get a free test on viral load, CD4+ cell count, CD 26/DPP IV levels and the results will be made available to the patients. The generated results will benefit other HIV patients in future on establishing the usefulness of the CD 26/DPPIV test.

Risks and discomforts of being in the study

The study will collect personal information from your child like the child’s name, HIV status, the use of ARVs, if he or she has suffered any other disease in the near past or if the child is tolerant or allergic to any drug. This information will be kept private. We will not keep the child’s name with the child’s sample or give it a code number that could identify your child. Therefore, nobody at the repository or who studies your child’s
sample will know it came from your child. All these information will be kept safely under lock and key or password protected computer files so that no unauthorized persons can access the information.

We will collect 3 ml of blood samples using a needle and a syringe/vacutainer tubes. This will be done immediately and after the child comes back after six months. The puncture of the needle may be uncomfortable and leave a bruise; the drawing of blood may cause pain where the needle enters the vein. Very rarely, some people experience light-headedness or possibly faint during this procedure. This will be reduced by allowing the child to take plenty of rest immediately after the procedure. There is also a small chance of infection occurring at the site where we take the child’s blood. This will be avoided by wiping the skin region with antiseptic like methylated spirit before drawing blood and covering the region with cotton wool soaked in the same antiseptic for some time after drawing the blood.

**Confidentiality/Privacy of information**

We will protect your child’s privacy in several ways. While KEMRI will keep your signed parental permission form, only the KEMRI ERC and JKUAT ethical review may request to see it. Your child’s name will be kept differently from its sample while any code number given to the sample will be in such a way that will not easily identify your child therefore nobody working with it will know it came from you.

At the end of the study, we plan to write a report. The report will not bear any information regarding your child’s personality, for example, the child’s name identity. We assure you of confidentiality of such information. Thus, we also need your permission to use the results for writing a report.

**Right to refuse or withdrawal**

You do not have to allow your child to take part in this research if you do not wish to do
so and this will not affect the child’s ability to receive treatment at the hospital. You may stop your child from participating at any time that you wish to without loosing any of the child’s right as a patient. Incase you allow your child to participate in this study; we will ask you to read and sign the parental permission form attached to this sheet. You will be given a copy of the signed and dated form to keep.

In case of any questions or concerns regarding this research, please contact the principal investigator by:

Ayub Kiprotich Maina

P.O Box 13096-00100,

Nairobi, Kenya.

0733937340

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact-anonymously if you wish-

The secretary,

KEMRI Ethics Review Committee,

P.O Box 54840-00200,

Nairobi, Kenya.

Tel: 020-2722541,

0722205901,

0733400003

Email address: erc@kemri.org.
Parental permission form

I the undersigned will like to confirm that as I give my child consent to participate in this study, it is with a clear understanding of the objective and condition of the study and with the recognition of my right to remove my child from the study.

I……………………………………., do hereby give consent to Dr/Mr ………………………………to include me in the proposed research and test. I have been given the necessary information to understand that there might be some risks involved in drawing of blood. I have also been assured that I can withdraw my permission at any time without penalty or loss of benefit due to my child. The proposal has been explained to me in the language I am conversant in.

Name of parent………………………………………………..

Parent’s signature………………………………………………

Date…………………………………………………………. Place…………………………

Name of Principal investigator……………………………………

Signature of principal investigator…………………………………

Date…………………………………………………………. Place…………………………

Kiswahili parental/guardian permission form

Nambari la Toleo: 2 Tarehe: 12/03/2013
Maelezo kwa watakaohusika katika utafiti lenye mada ifuatayo: Uchunguzi wa CD 26/Dipeptidyl peptidase kama unawezaashiria utumizi wa madawa ya ARVs kwa vijana walio na virusi vya HIV.

Jina la mtafiti mkuu.........................Ayub Kiprotich Maina

Jina la shirika.............................................JKUAT/KEMRI

Jina la kituo cha utafiti: Nyumbani diagnostic laboratory, Nairobi.

Lengo la utafiti: Tunaomba kushiriki kwa mtoto wako katika utafiti huu. Utafiti huu unatarajia kuchunguza wa kutumia kipengele cha mwili CD 26/DPPIV kutabiri kiwango cha virusi kwa wanaouguva HIV. Hii ni njia mpya ya bei nafuu na itasaidia katika uchunguzi wa wanaouguva virusi vya ukimwi kabla na baada ya kutumia madawa ya ARVs.

Maelezo ya utafiti

Faida ya utafiti

Watakaoshiriki wataweza kupimwa viwango vya viral load, CD4+ cells na CD 26/DPPIV na matokeo yote yatafunuliwa kwao. Matokeo haya yatasaidia wanaougu amukimwi kwa sasa na kwa siku za usoni kwa kufafanua utumizi wa kipengele hichi cha CD 26/DPPIV.

Hatari ya utafiti

Utafiti huu utahitaji maneno yanayowezakuwa ya faragha kutoka kwa mtoto wako kama vile jina lake, hali ya HIV, utumizi wa madawa ya ARVs, ikiwa ameugua ugonjwa yeyote hivi karibuni au kwa matokeo ya madawa fulani fulani. Maelezo haya yatawekwa kwa siri. Hatuweziweka jina lake na sampuli yake au kuipa sampuli yake kwa duili itakayomtambulisha. Kwa hivyo, yeyote atakayechunguza hawezijua ilitoka kwake. Maelezo haya yatawekwa vizuri kwa kufuli na ufunguo au liwewe kwa maneno ya siri katika tarakilishi.

Siri ya maelezo

Tutalinda maelezo tutakayopokea kutoka kwake kwa siri. Tutatumia njia zifuatazo. KEMRI italinda fomu hii ya ruhusa kutoka kwake na mtu mwingine yeyote hawezi kutazama isipokuwa tume la KEMRI ERC na JKUAT ERC. Hatuwezi kamwe kuweka sampuli yake kwa damu yake kwa kufuli na jina lake ama tuipatie nambari ambayo mtu yeyote atamatambua. Kwa hivyo hakuna yeyote atakayejua ilitoka kwake.
Wakati utafiti huu utafika mwisho, tunapanga kuandi ka ripoti kamili kuhusiana na matokeo yetu. Ripoti hii kamwe haitakuwa na maelezo yoyote kuhusiana na maneno yanayofaa kuwa siri kwake, kwa mfano, jina lake na chochote kile kinaweza kumtambulisha. Tunakuhakikishia kwamba maelezo kama hayo tuchunguza kwa siri. Kwa hivyo tunaomba ruhusa yako kutumia maetokeo tutakayapata kuandika ripoti kamilifu itakayosaidia wario na virusi vya HIV.

**Haki ya kukataa na kujiondoa kutoka kwa utafiti**

Sio lazima ukubali mtoto wako kushiriki katika utafiti huu ikiwa hauna nia ya kufanya hivyo, na chaguo lako kamwe halitakuzuza kupata matibabu katika hospitali. Unawezatoa kushiriki kwa mtoto wako katika utafiti huu bila kupoteza haki yake kama mgonjwa. Ikiwa utakubali motto wako kushiriki kwa utafiti huu, tunakuomba usome na uweke sahihi kwenye fomu la kuomba ruhusa kutoka kwako. Utapewa fomu hilo lililowekwa sahihi pamoja na tarehe yake ili uitunze.

Ikiwa una swali au tatizo lolote kuhusiana na utafiti huu, unaweza kuwasiliana na mtafiti mkuu kwa:

Ayub Kiprotich Maina

P.O Box 13096-00100,

Nairobi, Kenya.

0733937340

Ikiwa una tashwishi yeyote kuhusiana na haki zako kama mshirika wa utafiti huu au hajafurahia kitu chochote katika utafiti huu unawezawasiliana-ukitaka kwa siri:

Karani,

KEMRI Ethics Review Committee,
Sahihi ya Ruhusa

Ningependa kuhakikisha ya kwamba natoa ruhusa ya mtoto wangu kushiriki katika utafiti huu kwa sababu nimeelewa malengo na madhumuni ya utafiti huu na kwamba nina haki ya kumwondoa wakati wowote.

Mimi...............................................natoa ruhusa yangu kwa Dr/Mr.................................
ajumulise mwanangu katika utafiti unaopendekezwa. Nimepewa maelezo yote
ninayohitaji na kwamba kunawezakuwa na hatari wakati wa kutoa damu.
Nimehakikishiwa ya kwamba ninawezaondoa ruhusa yangu bila kutuhumiwa kwa
mwanangu au kumfanya apoteze faida yeyote anayopokea kwa sasa. Yote yanayohusiana
na utafiti huu umeelezewa kwangu kwa lugha ninayelewa.

Jina la mzazi....................... Sahihi..............................
Tarehe................................

Jina la mchunguzi....................... Sahihi..............................
Tarehe..............................
APPENDIX 2: GENERAL RESULTS IN ALL PATIENTS FOR CD 26/DPPIV, VIRAL LOADS AND CD 4+ CELL COUNTS

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<th>SEX</th>
<th>TIME BETWEEN BASELINE AND FIRST</th>
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<th>CD 26/DPPIV AFTER ART</th>
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**APPENDIX 3: ETHICAL APPROVAL LETTER**
KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205801, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

March 26, 2013

TO: Mr. AYUB KIPROTICH MAINA (PRINCIPAL INVESTIGATOR)
STUDENT No: TM305-1080/2011

THROUGH: DR. FRED OKOTH,
DIRECTOR, CVR,
NAIROBI

Dear Sir,

RE: SSC PROTOCOL No. 2344 (3RD RESUBMISSION) THE FEASIBILITY OF USING
Dipeptidyl peptidase IV/ CD 26 AS A SURROGATE AMRKER IN MONITORING ANTI-
RETROVIRAL DRUG THERAPY (VERSION 1.3 DATED 15TH MARCH 2013)

Reference is made to your letter dated March 07, 2013. The ERC Secretariat acknowledges receipt of the
revised proposal on 25 March 2013.

This is to inform you that at the Committee determines that the issues raised at the 210th meeting of the
KEMRI Ethics Review Committee held on 26th November 2012 are adequately addressed.

Consequently, the study is granted approval for implementation effective this 26th March 2013 for a
period of one year. Please note that authorization to conduct this study will automatically expire on March
26, 2014.

If you plan to continue data collection or analysis beyond this date, please submit an application for
continuation approval to the ERC Secretariat by February 12, 2014. The regulations require continuing
review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the
changes should not be initiated until written approval from the ERC is received. Please note that any
unanticipated problems resulting from the implementation of this study should be brought to the attention
of the ERC and you should advise the ERC when the study is completed or discontinued.

Work on this project may begin.

Sincerely,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health

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