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

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

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A Thesis Submitted in Partial Fulfillment for the Degree of Master of Science in Molecular Medicine in the Jomo Kenyatta University of Agriculture and Technology.

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……..

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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.

ACKNOWLEDGEMENTS

I would like to acknowledge the Kenya Medical Research Institute (KEMRI), Production Department for allowing me to use their labs to conduct this study. I would also like to acknowledge the Children of God Relief Institute (COGRI) for allowing me to use their Lea Toto Programme samples to conduct this study. My special acknowledgement also goes to the Department of Foreign Affairs and International Trade, Canada for providing funding for this research. I would finally like to acknowledge the National Commission for Science and Technology (NACOSTI) for also providing funding for this research.

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LIST OF ABBREVIATIONS/ACRONYMS

AIDS -Acquired Immune Deficiency Syndrome

ART -Anti-retroviral therapy

ARVs -Antiretroviral drugs

ADA -Adenosine Deaminase

CD -Cluster of Differentiation

DPPIV -Dipeptidyl peptidase IV

ELISA -Enzyme Linked Immunosorbent Assay

HIV -Human Imunodeficiency Virus

PBMC -Peripheral blood mononuclear cells

PCR -Polymerase Chain Reaction

TDF- Tenofovir Disoproxil Fumarate

NVP- Nevirapine

LPV/r- Lopinavir/Ritonavir also called Kaletra

AZT- Azidothymidine also called zidovudine

3TC- Lamivudine

ABC- Abacavir

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 investgate 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 sub set, 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).

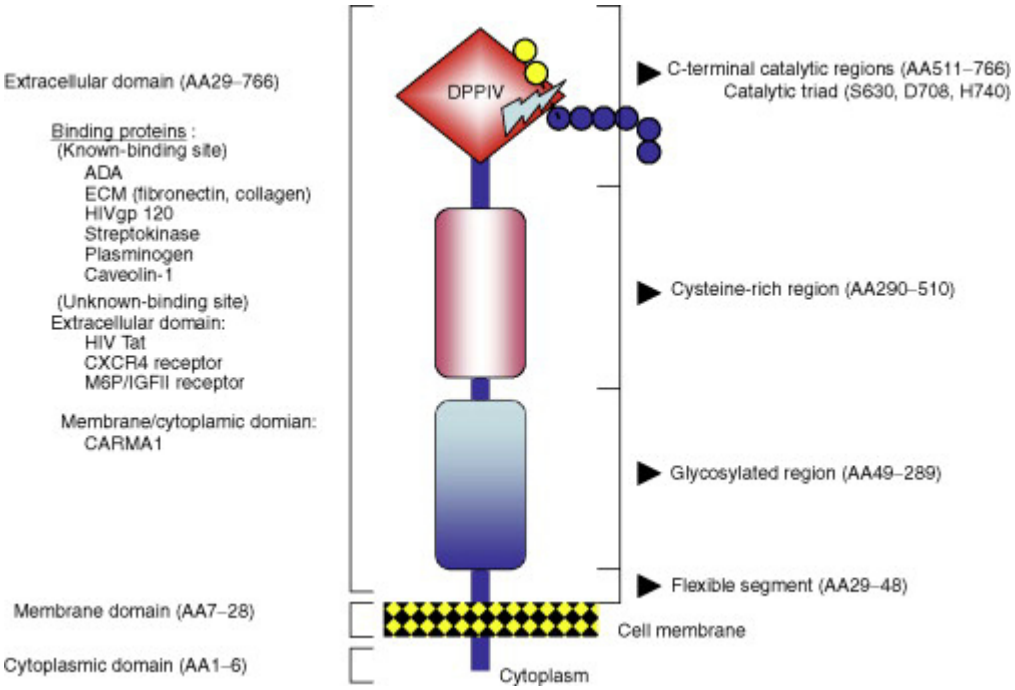


Figure 2.1: The structure of CD 26/DPPIV:

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 DPP IV 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 sialyation 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-erb*B-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

Lea Toto Centre	Kawangware	Kibera	Mukuru	Dandora	Kangemi	Zimmerman	Dagoretti	Kariobangi
Number of study participants	13	10	3	4	14	7	3	22

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 -80^0c 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 themophilus* 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 togther 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.

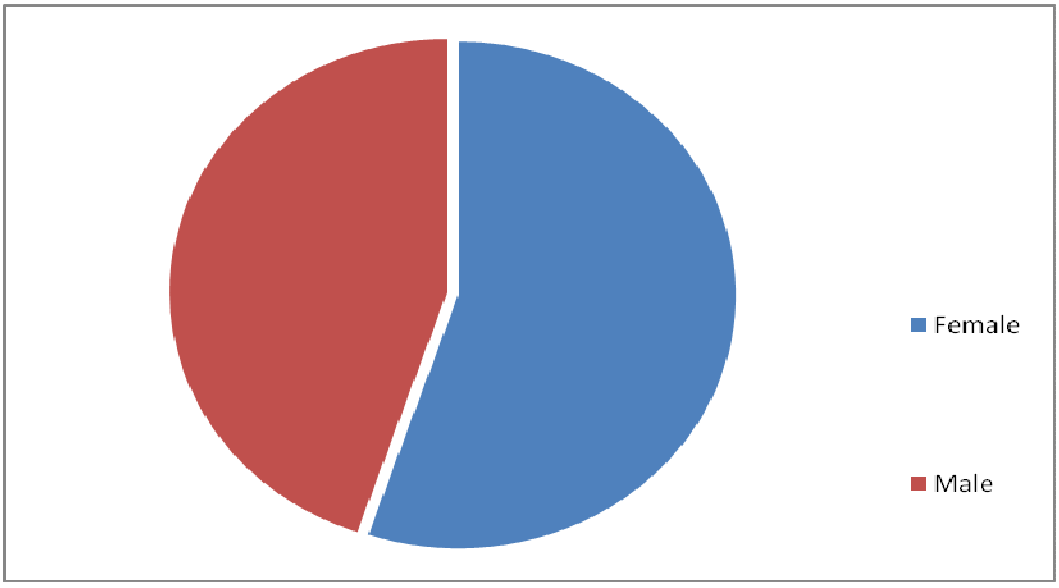


Figure 4.1: Pie chart showing the distribution of female study participants in relation to male study participants

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.

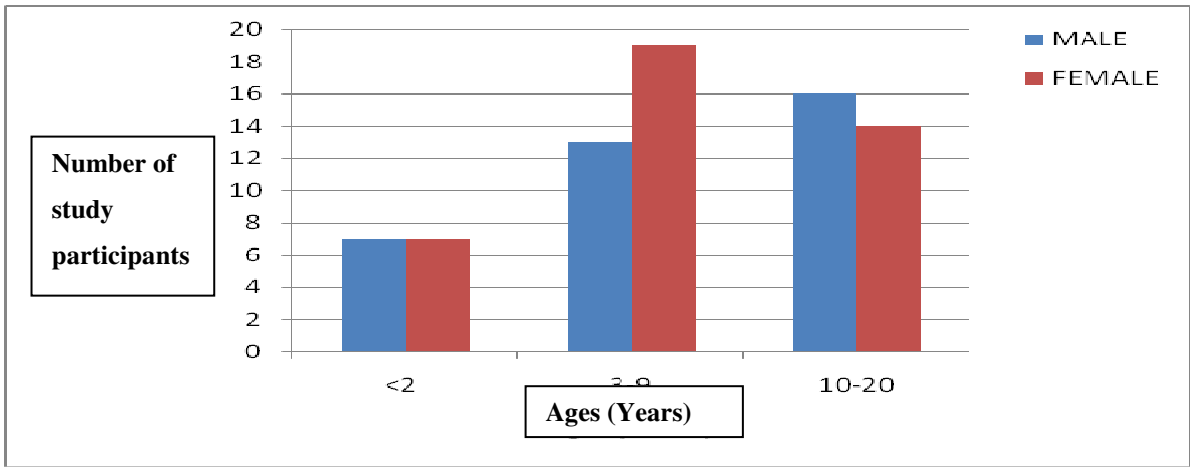


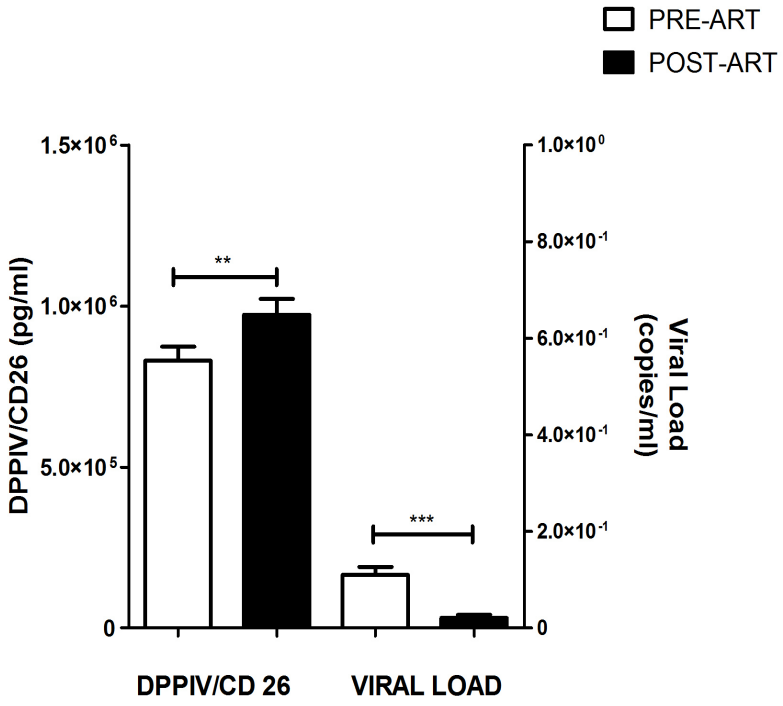
Figure 4.2: Histogram showing the age distribution of the study participants by gender in the Lea Toto programme in Nairobi, Kenya.

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

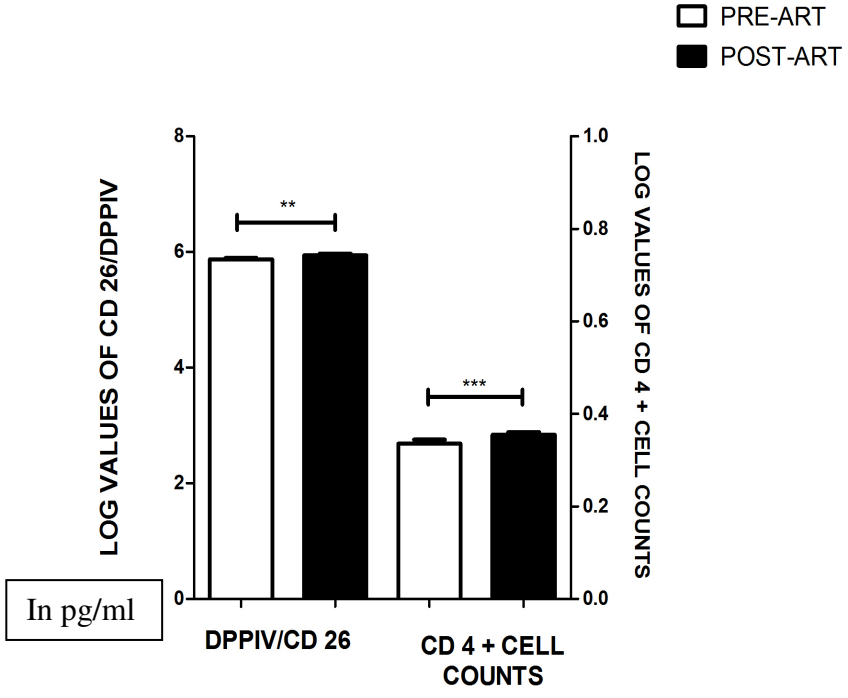
The average levels of DPPIV in the blood of study participants (n=76) before initiation of ART was 830800±438000 pg/ml which significantly rose after initiation of ART to 972800±606200 pg/ml (p=0.0017) (**Figure 4.3 a**). The average viral load in HIV infected study participants declined from 164600 ± 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 mm^3 which increased significantly after ART initiation to 963.8 ± 930.2 cells per mm^3 (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 CD 4+ 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:

a

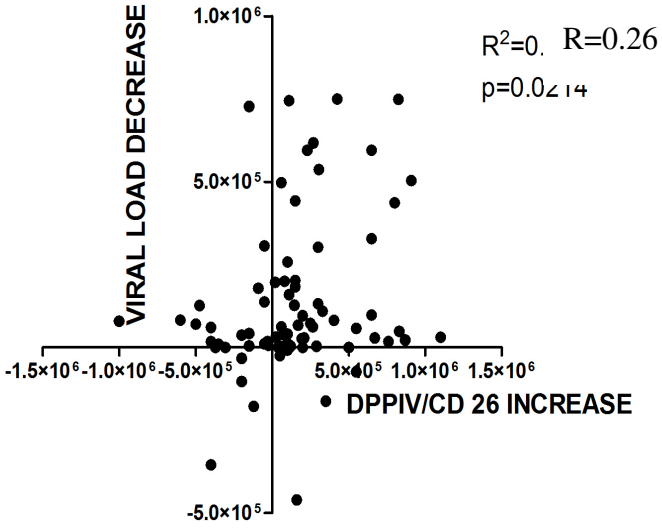

PRE-ART
POST-ART
1.5×10^6
1.0×10^6
5.0×10^5
0
DPPIV/CD26 (pg/ml)
1.0×10^0
8.0×10^-1
6.0×10^-1
4.0×10^-1
2.0×10^-1
0
Viral Load (copies/ml)
**
***
DPPIV/CD 26
VIRAL LOAD

b


PRE-ART
POST-ART
8
6
4
2
0
LOG VALUES OF CD 26/DPPIV
1.0
0.8
0.6
0.4
0.2
0.0
LOG VALUES OF CD 4 + CELL COUNTS
**
***
DPPIV/CD 26
CD 4 + CELL COUNTS

In pg/ml

c


VIRAL LOAD DECREASE
1.0×10⁶
5.0×10⁵
-5.0×10⁵
-1.5×10⁶ -1.0×10⁶ -5.0×10⁵
5.0×10⁵ 1.0×10⁶ 1.5×10⁶
DPPIV/CD 26 INCREASE
R²=0. R=0.26
p=0.0214

d

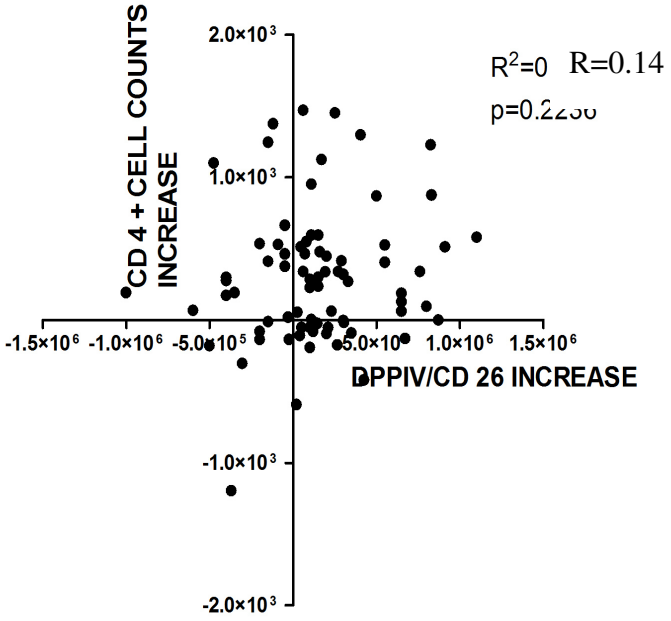

CD 4 + CELL COUNTS INCREASE
2.0×10³
1.0×10³
-1.0×10³
-2.0×10³
-1.5×10⁶ -1.0×10⁶ -5.0×10⁵
5.0×10⁵ 1.0×10⁶ 1.5×10⁶
DPPIV/CD 26 INCREASE
R²=0 R=0.14
p=0.2256

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

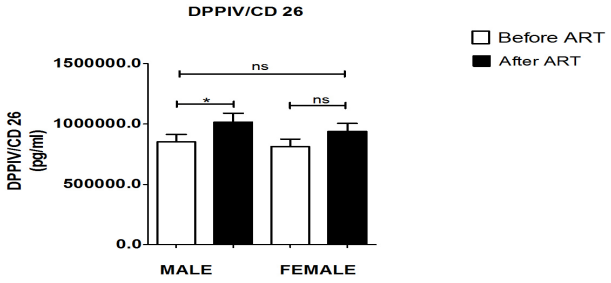
The levels of DPPIV in male study participants rose significantly from a mean of 851800 ± 485500 pg/ml before ART, to 1015000 ± 663000 pg/ml after ART (*p*=0.0112), and similarly in female the same rose from 813700 ± 480500 pg/ml before ART to 938700 ± 605500 pg/ml after ART (*p*=0.0526).When we compared 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

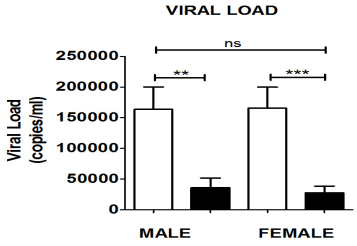
range of 163600 ± 205740 copies/ ml before ART, to a mean of 35900 ± 10610 copies/ml after ART (p=0.0031). In female study participants, the viral load levels declined significantly from a mean range of 165400 ± 140210 copies/ml before ART to a mean range of 27250 ± 8497 copies/ml after ART (p=0.0007). 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)**.

a


DPPIV/CD 26
Before ART
After ART
DPPIV/CD 26 (pg/ml)
1500000.0
1000000.0
500000.0
0.0
ns
*
ns
MALE
FEMALE

b


VIRAL LOAD
Viral Load (copies/ml)
250000
200000
150000
100000
50000
0
ns
**
***
MALE
FEMALE

c

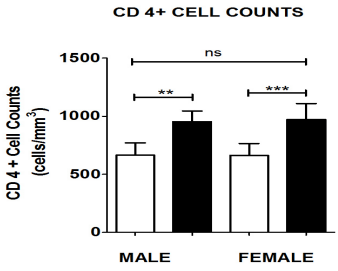

CD 4+ CELL COUNTS
CD 4 + Cell Counts (cells/mm3)
1500
1000
500
0
ns
**
***
MALE
FEMALE

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

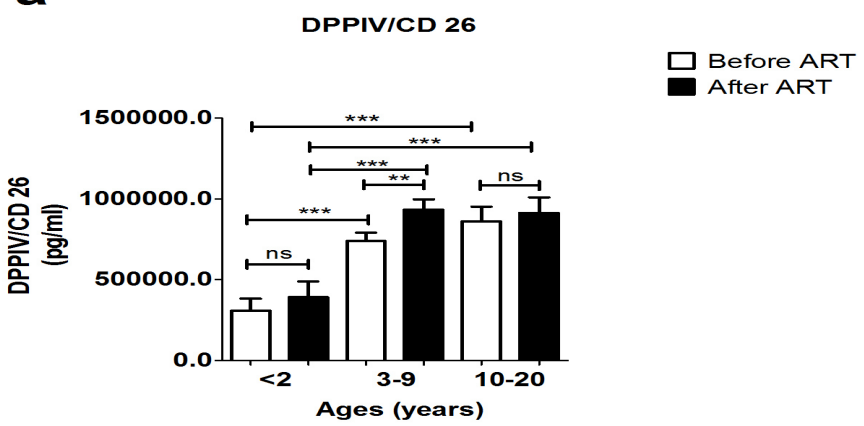
with ART. When the change in viral load levels before and after ART was compared between individuals below 2 years of age to those study participants between ages 10-20 using Mann-Whitney test respectively, p was 0.0003 before ART and p=0.001 after ART which are both statistically significant indicating 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
DPPIV/CD 26 (pg/ml)
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1000000.0
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ns
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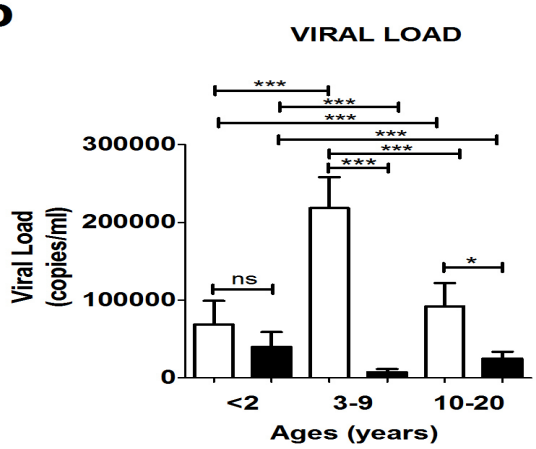
ns
<2
3-9
10-20
Ages (years)

b


VIRAL LOAD
Viral Load (copies/ml)
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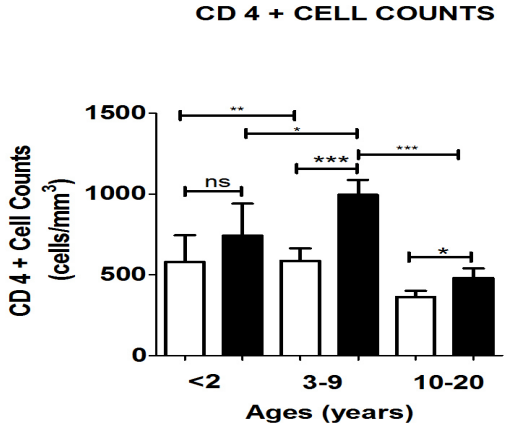
3-9
10-20
Ages (years)

c


CD 4 + CELL COUNTS
CD 4 + Cell Counts (cells/mm3)
1500
1000
500
0
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*
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<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.

	DPPIV before ART in pg/ml	**DPPIV after ART in pg/ml**	**viral load before ART in copies per ml**	**Viral load after ART in copies per ml**	**CD4+ cell count before ART in cells per mm^3**	**CD4+ cell counts after ART in cells per mm^3**
Study participants of ages 10-20 years	949733.3	1005333	101108.7	27133.73	399.7	529.9
Study participants of 3-9 years	740757.6	933333.3	218446.6	7364.061	587.45	994.42
Study participants below 2 years of age	784615.4	998076.9	174246.7	100604	1467.85	1887.46

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 billiary 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 acess 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 alternatve 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 thresults 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 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……

Name of Principal investigator……

Signature of principal investigator…….

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.....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 kiwango 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

Watakaoshiriki katika utafiti huu ni vijana walio na virusi vya HIV ambao damu yao itapimwa kuchunguza kiwango cha CD 26/DPPIV kabla na baada ya miezi sita tangu waanze kutumia madawa ya ARVs. Baada ya ruhusa kutoka kwa watakaoshiriki, mililita tatu ya damu yao itatolewa na kuwekwa kwa chupa maalumu iliyo na kemikali ya kuweza kufanya damu isigandamane. Vijichupa hivyo vilivyo na damu vitawekwa kwenye kifaa maalum kilicho na baarafu kisha kipelekwe kwenye zahanati kufanyiwa uchunguzi. Katika zahanati, sampuli hizo za damu zitahifadhiwa kwenye friji wakati uchunguzi wao utakuwa unaendelea**.**

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. Hatuweziweka jina lako na sampuli yako au kuipa sampuli yako kodi itakayokutambulisha. Kwa hivyo, yeyote atakayechunguza hawezijua ilitoka kwako. Maelezo haya yatawekwa vizuri kwa kufuli na ufunguo au liwekwe kwa maneno ya siri katika tarakilishi.Tutachukua mililita tatu ya sampuli ya damu kutoka kwako kwa kutumia sindano. Sampuli ya kwanza itachukuliwa kwa sasa na nyingine ukirudi baada ya miezi sita. Unawezahisi uchungu kidogo na kuvimba kwa sehemu iliyodungwa. Mara chache, watu wengine huisi kizunguzungu au kuzimia wakati wa kitendo hichi cha kutoa damu, lakini kwa nadra kabisa. Shida hii itakingwa na kupumzika baada ya kutolewa damu.Vilevile, kuna uwezekanao mdogo unawezapata maambukizi kwa sehemu tutakayotolewa damu. Shida hii itakingwa kwa kupanguza sehemu ya kutolewa damu

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.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 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. Tunakuhakikishia kwamba maelezo kama hayo tuyaweka 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 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 kunawezakuwa na hatari wakati wa kutoa damu. Nimehakikishiwa ya kwamba ninawezaondoa ruhusa yangu bila kutuhumiwa au kupoteza faida yeyote ninayopokea kwa sasa. Yote yanayohusiana na utafiti huu umeelezewa 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

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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

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Confidentiality/Privacy of information

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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.

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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 kiwango 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

Watakaoshiriki katika utafiti huu ni vijana walio na virusi vya HIV ambao damu yao itapimwa kuchunguza kiwango cha CD 26/DPPIV kabla na baada ya miezi sita tangu waanze kutumia madawa ya ARVs. Baada ya ruhusa kutoka kwa watakaoshiriki, mililita tatu ya damu yao itatolewa na kuwekwa kwa chupa maalumu iliyo na kemikali ya kuweza kufanya damu isigandamane. Vijichupa hivyo vilivyo na damu vitawekwa kwenye kifaa maalum kilicho na baarafu kisha kipelekwe kwenye zahanati kufanyiwa uchunguzi. Katika zahanati, sampuli hizo za damu zitahifadhiwa kwenye friji wakati uchunguzi wao utakuwa unaendelea.

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. Hatuweziweka jina lako na sampuli yako au kuipa sampuli yako kodi itakayokutambulisha. Kwa hivyo, yeyote atakayechunguza hawezijua ilitoka kwako. Maelezo haya yatawekwa vizuri kwa kufuli na ufunguo au liwekwe kwa maneno ya siri katika tarakilishi.Tutachukua mililita tatu ya sampuli ya damu kutoka kwako kwa kutumia sindano. Sampuli ya kwanza itachukuliwa kwa sasa na nyingine ukirudi baada ya miezi sita. Unawezahisi uchungu kidogo na kuvimba kwa sehemu iliyodungwa. Mara chache, watu wengine huisi kizunguzungu au kuzimia wakati wa kitendo hichi cha kutoa damu, lakini kwa nadra kabisa. Shida hii itakingwa na kupumzika baada ya kutolewa damu.Vilevile, kuna uwezekanao mdogo unawezapata maambukizi kwa sehemu tutakayotolewa damu. Shida hii itakingwa kwa kupanguza sehemu ya kutolewa damu 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.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 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. Tunakuhakikishia kwamba maelezo kama hayo tuyaweka 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 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 kunawezakuwa na hatari wakati wa kutoa damu. Nimehakikishiwa ya kwamba ninawezaondoa ruhusa yangu bila kutuhumiwa au kupoteza faida yeyote ninayopokea kwa sasa. Yote yanayohusiana na utafiti huu umeelezewa 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 uwezekano wa kutumia kipengele cha mwili CD 26/DPPIV kutabiri kiwango 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

Watakaoshiriki katika utafiti huu ni vijana walio na virusi vya HIV ambao damu yao itapimwa kuchunguza kiwango cha CD 26/DPPIV kabla na baada ya miezi sita tangu waanze kutumia madawa ya ARVs. Baada ya ruhusa kutoka kwa watakaoshiriki, mililita tatu ya damu yao itatolewa na kuwekwa kwa chupa maalumu iliyo na kemikali ya kuweza kufanya damu isigandamane. Vijichupa hivyo vilivyo na damu vitawekwa kwenye kifaa maalum kilicho na baarafu kisha kipelekwe kwenye zahanati kufanyiwa uchunguzi. Katika zahanati, sampuli hizo za damu zitahifadhiwa kwenye friji wakati uchunguzi wao utakuwa unaendelea .

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 kutoka kwa mtoto wako kama vile jina lake, hali ya HIV, utumizi wa madawa ya ARVs, ikiwa ameugua ugonjwa yeyote hivi karibuni au ikiwa mwili wake haielewani na madawa fulani fulani. Maelezo haya yatawekwa kwa siri. Hatuweziweka jina lake na sampuli yake au kuipa sampuli yake kodi itakayomtambulisha. Kwa hivyo, yeyote atakayechunguza hawezijua ilitoka kwake. Maelezo haya yatawekwa vizuri kwa kufuli na ufunguo au liwekwe kwa maneno ya siri katika tarakilishi.Tutachukua mililita tatu ya sampuli ya damu kutoka kwake kwa kutumia sindano. Sampuli ya kwanza itachukuliwa kwa sasa na nyingine akirudi baada ya miezi sita. Unawezahisi uchungu kidogo na kuvimba kwa sehemu iliyodungwa. Mara chache, watu wengine huisi kizunguzungu au kuzimia wakati wa kitendo hichi cha kutoa damu, lakini kwa nadra kabisa. Shida hii itakingwa na kupumzika baada ya kutolewa damu.Vilevile, kuna uwezekanao mdogo anawezapata maambukizi kwa sehemu tutakayotolewa damu. Shida hii itakingwa kwa kupanguza sehemu ya kutolewa damu 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 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 ya damu yake ikiwa na jina lake ama tuipatie nambari ambayo mtu yeyote atamtambua. Kwa hivyo hakuna yeyote atakayejua ilitoka kwake.

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 kwake, kwa mfano, jina lake na chochote kile kinaweza kumtambulisha. Tunakuhakikishia kwamba maelezo kama hayo tuyaweka 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 ukubali mtoto wako kushiriki katika utafiti huu ikiwa hauna nia ya kufanya hivyo, na chaguo lako kamwe halitakuzuia 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,

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 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..... ajumulishe 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 ninayoelewa.

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

PATIENT CODE	CENTRE	AGE	SEX	TIME BETWEEN BASELINE AND FIRST	CD 26/DPPIV BEFORE ART	CD 26/DPPIV AFTER ART	VIRAL LOAD BEFORE ART	VIRAL LOAD AFTER ART	CD 4 BEFORE ART	CD 4 AFTER ART

				SAMPLE						
AY001	Kariobangi	10	F	14	192000	385000	27713	638	361	700
AY002	Kawangware	7	F	6	480000	625000	135453	8174	838	816
AYOO3	Kariobangi	10	M	7	400000	450000	169032	193336	86	33
AY004	Kibera	10	F	6	600000	700000	48371	55843	412	698
AY005	Mukuru	3	F	8	740000	850000	159828	50	352	1304
AY006	Kariobangi	8	F	8	970000	1800000	49136	50	252	1129
AY007	Kangemi	17	F	5	625000	600000	7038	81	815	680
AY008	Kawangware	8	F	8	550000	610000	62578	50	582	923
AY009	Zimmerman	3	F	3	700000	500000	36503	68652	398	319
AY010	Mukuru	9	F	8	1400000	1500000	11339	50	314	262
AY011	Kariobangi	2	F	7	750000	1300000	37855	111314	1080	1486
AYO12	Zimmerman	6	M	9	810000	1100000	4329	756	1019	1435
AY013	Kangemi	2	F	3	800000	1900000	31151	50	799	1381
AY014	Zimmerman	1	F	13	740000	800000	497652	67	2810	4280
AY015	Kariobangi	20	F	5	390000	600000	99677	70515	259	206
AY016	Zimmerman	14	F	12	750000	700000	137471	50	412	1076
AY017	Kibera	6	F	1	735000	1000000	65812	3159	865	692

AY018	Dagoretti	6	F	6	900000	1100000	96255	50	415	862
AY019	Kangemi	16	F	7	1050000	1090000	439	184	538	430
AY020	Kariobangi	4	F	18	395000	800000	81916	152	650	1947
AYO21	Kariobangi	7	M	6	900000	810000	181678	2725	593	1123
AY022	Kawangware	11	F	7	1400000	1000000	17826	50	453	626
AY023	Kangemi	4	F	6	395000	700000	537319	457	999	984
AY024	Kawangware	13	F	9	1300000	1500000	50	50	361	265
AY025	Kibera	2	F	6	750000	1400000	328377	46	1294	1422
AYO26	Kangemi	1	M	13	700000	580000	622	178467	38	1414
AY027	Zimmerman	3	F	9	600000	125000	126750	50	15	1116
AY028	Kangemi	11	F	6	2200000	1200000	80339	967	191	385
AY029	Kawangware	12	F	11	1350000	1420000	38823	50	492	958
AY030	Kariobangi	1	F	9	1100000	900000	4156	106574	3214	3079
AY031	Zimmerman	9	F	6	470000	700000	595546	193	319	381
AY032	Zimmerman	4	F	7	1300000	900000	60572	50	797	1075
AY033	Kawangware	6	F	8	1050000	1200000	202413	40	204	505
AY034	Kariobangi	9	F	3	800000	750000	307114	50	341	805
AY035	Kariobangi	16	F	7	450000	600000	442434	50	126	364

AY036	Kawangware	6	F	11	610000	1370000	18098	50	374	715
AY037	Kariobangi	10	F	6	580000	1250000	36403	7692	436	308
AY038	Kariobangi	4	F	3	475000	900000	750000	50	1405	987
AY039	Dagoretti	2	F	6	700000	300000	68722	422436	21	321
AY040	Kibera	9	F	2	800000	600000	38978	1691	454	991
AY041	Kariobangi	10	F	7	1600000	1950000	5542	167435	328	237
AY042	Kangemi	12	F	12	1000000	500000	149205	79047	246	66
AY043	Kariobangi	10	F	2	820000	940000	9153	5573	145	67
AY044	Kariobangi	1	F	7	900000	750000	750000	22201	1811	3058
AY045	Kawangware	14	F	12	385000	410000	41703	9667	225	280
AY046	Kariobangi	3	F	3	375000	1200000	750000	724	1439	2667
AY047	Kawangware	7	M	9	270000	380000	750000	5576	258	855
AY048	Kariobangi	12	M	7	250000	520000	750000	132300	629	970
AY049	Kibera	11	M	4	980000	1850000	23418	670	506	506
AY050	Kawangware	1	M	9	740000	785000	4762	50	1275	1788
AY051	Dandora	3	M	11	800000	950000	183238	50	616	1213
AY052	Kawangware	6	M	6	330000	500000	67278	50	261	1386
AY053	Mukuru	3	M	5	720000	970000	72757	50	17	1469

AY054	Kangemi	8	M	2	800000	1100000	340183	37980	14	11
AY055	Dandora	4	M	8	690000	1600000	503671	61	925	1438
AY056	Kangemi	13	M	3	1300000	700000	83044	663	475	544
AY057	Dandora	13	M	3	890000	1000000	8621	50	482	486
AY058	Kariobangi	2	M	4	600000	760000	672	460172	1254	1732
AY059	Kariobangi	12	M	7	1100000	950000	42298	50	135	547
AY060	Kawangware	8	M	7	1250000	1550000	132108	50	116	437
AY061	Kangemi	2	M	6	600000	700000	258718	306	1612	1420
AY062	Dandora	9	M	7	1400000	1350000	11760	670	426	804
AY063	Kangemi	11	M	6	950000	600000	10139	169	670	863
AY064	Kariobangi	11	M	4	1250000	1900000	595546	193	319	381
AY065	Kariobangi	12	M	10	1550000	1400000	28873	23963	451	440
AY066	Kangemi	2	M	6	450000	1100000	104363	6222	1831	2019
AY067	Kibera	8	M	5	770000	1100000	109940	62	76	347
AY068	Kibera	17	M	5	1400000	1500000	40230	50	463	691
AY069	Kariobangi	3	M	6	690000	710000	306400	109865	1286	695
AY070	Kangemi	4	M	8	670000	750000	201068	1071	1154	1703
AY071	Kangemi	1	M	10	350000	1150000	436825	203	781	878

AY072	Kibera	10	M	12	1300000	1800000	50	50	335	1205
AY073	Dagoretti	11	M	7	850000	1400000	58559	926	486	1012
AY074	Kariobangi	14	M	17	750000	445000	50	50	912	609
AY075	Kawangware	14	M	10	830000	800000	81214	63650	243	264
AY076	Kibera	1	M	8	1620000	1250000	50	50	2874	1679

APPENDIX 3: ETHICAL APPROVAL LETTER

KENYA MEDICAL RESEARCH INSTITUTE

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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

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. Box 54628
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 25, 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

CENTRE FOR VIRUS RESEARCH
02 APR 2013
P.O. Box 54628 - NAIROBI