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Research Article

The Potential for DPPIV/CD26 usage as a surrogate marker for Antiretroviral Therapy Efficacy in HIV Infected populations

Ayub K. Maina [a,*], Joseph K. Mbugua [a], Adriana Trajtman [b], Yoav Keynan [b], Robert W. Omange [b] Annie Panikulam [c], Rachel N. Musoke [c,d], James Kimotho [e], Missiani Ochwoto [e], Daniel Kariuki [a], and Elijah M. Songok [a,b,e]

[a] *Institute of Tropical Medicine & Infectious Diseases, Jomo Kenyatta University of Agriculture & Technology, Kenya*
[b] *Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada*
[c] *Children of God Relief Institute - Nyumbani Diagnostic Laboratory, Nairobi*
[d] *Department of Paediatrics, University of Nairobi, Kenya*
[e] *Kenya Medical Research Institute (KEMRI), Nairobi, Kenya*

* **Corresponding author:** Institute of Tropical Medicine & Infectious Diseases, Jomo Kenyatta University of Agriculture & Technology, P.O. Box 62000-00200, Nairobi, Kenya. **Tel:** +254-20-2027952, **Email:** ayubkmaina@yahoo.com

Background: Human Immunodeficiency Virus (HIV) viral load and CD4+ cell counts are the most commonly used markers for monitoring efficacy of anti-retroviral therapy (ART) in HIV infected individuals. The high cost of viral load monitoring limits its usage in resource limited countries, often leaving the use of CD4+ T cell counts as the only alternative. Though cheaper and more readily available, CD4+ cell counts as a measure of detecting treatment failure, is an unreliable predictor of disease progression. Hence, there is a need for more sensitive alternative, but less costly techniques for detecting treatment failure which can be used in resource limited settings.

Objective: To evaluate the feasibility of using plasma CD26/Dipeptidyl peptidase IV (DPPIV) as a novel marker for clinical evaluation of treatment efficacy in HIV infected children.

Method: Blood samples collected from HIV+ children (n=76) before and after initiation on ART, were assessed for HIV RNA (viral load), CD4+ T-cell count and DPPIV/CD26 levels. Viral load levels were analyzed using Roche Amplicor HIV-1 Monitor Test kit; CD4+ T-Cell Counts were analyzed using BD FACS Calibur flow cytometer while DPPIV/CD 26 levels were analyzed using Human DPPIV/CD26 Quantikine ELISA kit (R&D Systems, Minneapolis MN).

Results: The plasma DPPIV/CD26 levels increased significantly in children after ART initiation (p = 0.017), while the viral load levels declined after ART initiation with subsequent CD4+ cell counts increase. The DPPIV/CD 26 increase positively correlated with viral load decrease while negatively correlating to the CD4+ cell count increase.

Conclusion: These findings demonstrate an inverse relationship between DPPIV/CD26 levels and HIV viral load and the direct proportionality of CD4+ Cell counts and DPPIV/CD26 levels, suggesting potential for use of DPPIV/CD26 as a surrogate marker for evaluating HIV disease progression in children receiving anti-retroviral therapy.

Key words: CD26/Dipeptidyl peptidase IV (DPPIV), ELISA, Surrogate marker, Viral Load, CD4 Count, antiretroviral.

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1. Introduction

Since its emergence, HIV/AIDS has remained as one of the worst scourges to afflict humanity. (Gottlieb et al, 1981; Barré-Sinoussi et al, 1983; Schüpbach et al, 1985; Gallo, 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 report, 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 2014 (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 lifelong 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 (Reeves and Piefer, 2006). $CD4^+$ 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; Jain et al, 2013; 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 (UNAIDS, 2013). 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 (Katzenstein et al, 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, 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/CD26 levels could inversely correlate to HIV viral load, and directly correlate with CD4+ T cell levels in adult infected persons (Ohtsuki et al, 2001). This supported the premise that increased expression of DPPIV may be immunologically relevant to the course of HIV disease (Wrenger et al, 1997; Dong and Morimoto, 1996). Additionally, a study conducted in commercial sex workers (CSWs) from Kenya, found a higher expression of the enzyme Dipeptidyl peptidase IV/CD26 in the peripheral blood of HIV exposed seronegative Female Sex Workers (FSW) (Songok et al, 2010). These findings suggest that, higher DPPIV/CD26 levels may have a protective role against HIV acquisition among females' commercial sex workers in Kenya. CD26 is a 110 kDa protein that has dipeptidyl peptidase IV activity which 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, 2001; Havre et al, 2008; Cordero et al, 2009; Iwaki-Egawa et al, 1998). Soluble DPPIV or sCD26 is enzymatically active in biological fluids including peripheral blood (Havre et al, 2008; Cordero et al, 2009; Iwaki-Egawa et al, 1998).

In this study, we determined the relationship between the plasma levels of DPPIV/CD26, HIV viral load and CD4+ T cell counts in HIV infected individuals, as a means of evaluating the potential for use of DPPIV/ CD26 levels as a surrogate marker for HIV disease progression in children receiving ART.

2. Methodology

2.1 Study Site

This study was conducted in a group of children 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

in the low income areas of Nairobi, Kenya. The sampling was done at the various Lea Toto Centres namely: Kawangware, Kibera, Dagoretti, Mukuru, Kariobangi, Zimmerman, Dandora and Kangemi. 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).

2.2 Study design and study population

The study design was a comparative before and after study.

The study participants were all HIV positive children 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 children between the ages of 1 year to 20 years from all the Lea Toto centres in Nairobi were recruited randomly in this study using simple random sampling method.

2.3 Care Services provided under the Lea Toto Programme

Children 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 children are also offered routine prophylaxis and treatment for opportunistic infections. The nutritional status is also closely monitored, and parents or care-givers are advised on a balanced diet.

During the period of study, the Kenyan ART guideline for children was that all children below 10 years old, ART is started automatically regardless of the CD 4+ cell counts but for children 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 older children or 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.

2.4 Sample collection and preparation

The first sample was collected before ART initiation and second sample collected an average of 7.2 months after ART initiation. A total of 152 samples were 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 separated by centrifuging at 1500 rpm for 7 minutes.

The plasma was used for HIV viral load quantification then refrigerated at -80^0 C for a month and later used for soluble DPPIV/CD26 quantification.

2.5 DPPIV/CD26 quantification by ELISA.

Soluble DPPIV/CD26 plasma levels, was quantified using Human DPPIV/CD26 Quantikine ELISA kit (R&D Systems, Minneapolis MN) according to the manufacturer's recommendations. DPPIV/CD26 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, 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).

2.6 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, 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, 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. 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.

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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; low-positive and high-positive tested in each assay run provided with the kit. The kit does not specify the exact identity of the controls.

2.7 $CD4^+$ 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.

2.8 Data Analysis

Data analysis was performed using the Graphpad Prism version 5.0. Comparison of general DPPIV/CD26, viral loads and CD4+ T cell counts before and after initiation of ART, was done using paired t-tests. Correlational analysis of DPPIV/CD26 levels with viral load and CD4+ T cell counts was conducted using Spearman'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 children 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.

2.9 Ethical considerations

Ethical approval for this study was obtained from the Kenya Medical Research Institute Ethics committee 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.

3. Results

This study included 76 children- 34 (44.7%) male and 42 (55.3%) female-with equal distribution of sexes. The average ages per sex were, 7.53 and 7.81 for male and female, respectively, with a cumulative average age of 7.8 years for both sexes. Fourteen children were below 2 years, with equal number of males and female, 32 of the children were aged 3-9 (13 female and 19 male) and 30 were adolescents aged between 10-20 (16 females and 14 males) . The distribution of the children sampled from the eight Lea Toto programme centres was as follows; 13 from Kawangware (8 females and 5 males), 9 from Kibera (5 males and 4 females), 3 from Mukuru (2 females and 1 male), 4 from Dandora (all male), 14 from Kangemi (7 females and 7 males), 7 from Zimmerman (6 females and 1 male), 3 from Dagoretti (2 females and 1 male) and finally 23 were from Kariobangi (14 females and 9 males).

HIV infected children had DPPIV/CD26 levels corresponding to CD4+ T cell counts but inversely correlating to HIV viral loads

First we sought to establish the levels of DPPIV/CD26, previously shown to positively correlate with $CD4^+$ T cell counts, and negatively with HIV viral load in children. Moreover, DPPIV/CD26 levels have been associated with improved outcomes in HIV infected individuals and reduced susceptibility to HIV in HESN female CSWs in Nairobi (16, 17).We hypothesized that DPPIV/CD26 plasma levels would be directly proportional with levels of CD4 T cells but inversely to viral loads. To test this, we quantified the levels of soluble DPPIV/CD26, HIV viral load in the plasma, and CD4+ T cells counts from the whole blood of HIV infected children. We observed that, the average levels of DPPIV/CD26 in the blood of children (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 1a**). The average viral load in HIV infected children 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 1b)** .

Next we compared the relationship between increases in DPPIV/CD26 levels and declines in viral loads following ART initiation in children using Spearman's correlational analysis which was statistically significant showing that this inverse proportionality relationship is significant (p= 0.0246) **(Figure 1c)**. The correlation between DPPIV/CD26 increase and the $CD4^+$ cell counts increase using the same Spearman's correlational coefficient was however not significant with a (p =0.2236) **(Figure 1d)**. This showed that DPPIV/CD26 levels in HIV infected children before and after the initiation of ART was

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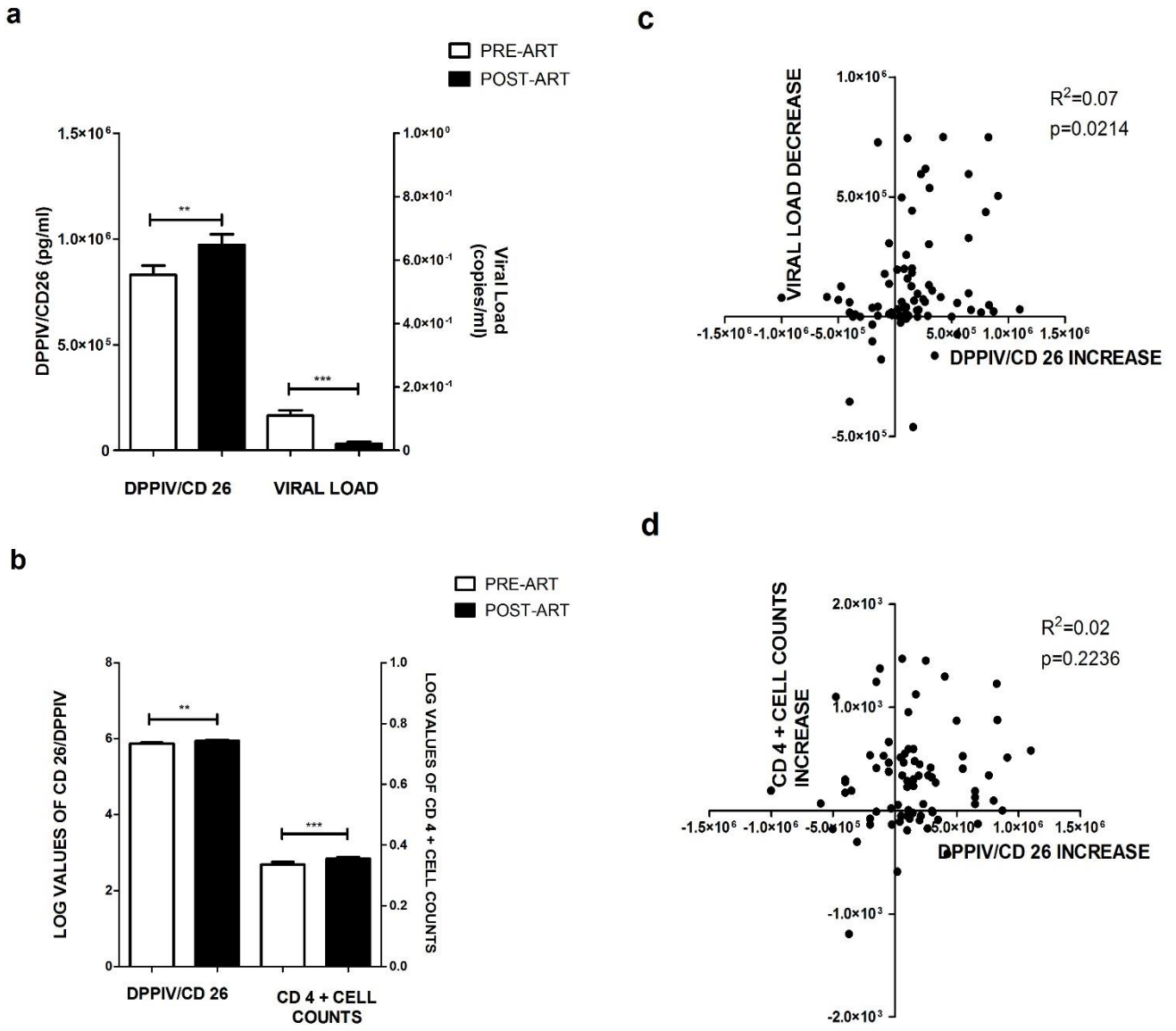
inversely proportional to the viral load levels but directly proportional to the CD4+ T cell counts, corresponding to what we had previously hypothesized. (Songok et al, 2010; Ohtsuki et al, 2000). **Table 1** showing the average DPPIV/CD26, viral load levels and CD4+ Cell counts in the various age specific groups before and after ARV use.

Influence of sex and age on DPPIV/CD26 levels in HIV infected children on ART

Next we sought to determine if sex and age of HIV positive children, prior to and following initiation of ART influenced the levels of DPPIV/CD26. Here we compared the levels of DPPIV/CD 26, viral loads, CD 4+ cell counts between groups of children categorized based on age and gender.

The levels of DPPIV/CD26 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 children 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/CD26 with gender.



a) *The DPPIV/CD26 levels (pg/ml) as quantified using ELISA while HIV viral loads were quantified using RT-PCR, before and after initiation of ART in HIV infected children compared to viral load levels (copies/ml).*
b*) The DPPIV/CD26 levels (log pg/ml) compared to the CD4+ Cell counts (log cells/mm³) before and after the use of ARVs. The changes in DPPIV/CD26 levels, HIV viral loads and CD4+ cell counts (before and after ART) were statistically analyzed using paired t-test. Error bars represent standard error of mean (S.E.M), and * represents p < 0.05, ** p <0.01 and *** is p <0.001.*
c *and* ***d)*** *Spearman's correlational analysis of DPPIV/CD26 levels and HIV viral loads or CD4+ T cell counts. Spearman's coefficient R^2 indicates the strength of associations and p-values represent the significance of the associations.*

Figure 1. DPPIV/CD26 in relation to viral load and CD4+ cell counts.

Table 1: The average DPPIV/CD26, viral load and CD 4+ Cell counts in adolescents, children of ages 3-9 and in infants.

	CD26 before ART in pg/ml	CD26 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
Adolescents 10-20 years	949733.3	1005333	101108.7	27133.73	399.7	529.9
Children 3-9 years	740757.6	933333.3	218446.6	7364.061	587.45	994.42
Infants below 2 years of age	784615.4	998076.9	174246.7	100604	1467.85	1887.46

Table 1 *shows the general mean increases in DPPIV/CD26 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.*

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 children before and after ART (Mann-Whitney p=0.9875).

The CD4+ T cell counts before and after ART in male children 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 children, 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.

In children below 2 years of age, DPPIV/CD26 levels before (Mean= 784615.4 ± 200000 pg/ml) and after (mean= 998076.9 ± 520000 pg/ml) ART, remained unchanged (p=0.15). However, in children between ages of 3-9 years, DPPIV/CD26 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 adolescents between the ages of 10-20, DPPIV/CD26 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/CD26 levels in children below 2 years of age to children 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/CD 26 between these 2 age groups before ART. When we compared the change in DPPIV/CD 26 after ART between the children below 2 years of age to those between ages 3-9,p<0.0001 which is statistically significant indicating a difference in DPPIV/CD 26 between the children of below 2 years to those between 3-9 years after ART. When the same was done between children below 2 years to adolescents 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/CD 26 in children of ages 3-9 was compared to the change in DPPIV/CD 26 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/CD 26 between ages 3-9 and ages 10-20 before and after ART respectively.

Viral load levels in infants 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 children 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 ARVs (p<0.0001). Similarly, the viral load levels in adolescents 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 children 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 children 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 children 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 children 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 children below 2 years of age to those 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 children 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 children of ages 3-9 to those 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 children of ages 3-9 to those between 10-20 before or without ART but there is no

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significant difference in viral load levels between these two age groups after ART.

The CD4+ T cell counts remained unchanged in infants 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 adolescents 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 children below 2 years to children 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 children 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 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.

4. Discussion

DPPIV/CD26 is an ectoenzyme that cleaves penultimate proline or alanine residues from the N-terminal of dipeptides or polypeptides. DPPIV/CD26 exists either in a membrane bound or soluble isoform, the latter circulates in the peripheral blood (Ohnuma et al, 2008). DPPIV/CD26 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, we observed a higher expression of DPPIV/CD26 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/CD26 in HIV infected children and young adults, could be used a surrogate marker for disease progression

The present study, demonstrated for the first time that the levels of DPPIV/CD26 in HIV infected children and young adults, was inversely proportional to HIV viral load but directly proportional to CD4+ T cells counts. In multiple instances, DPPIV/CD26 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 0-20 years, plateauing at between 10-20 years. Anti-retroviral therapy caused significant declines in DPPIV/CD26 levels in males but not females. Often, the levels of DPPIV/CD26 remained unaffected by ART. The exception was in children aged 3-9 years, who had higher levels of DPPIV/CD26 following ART initiation. Based on the same age stratification, the HIV viral load levels were highest in children 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 children between 3-9 years and in young adult between10-20 years, but failed to do the same in children below 2 years of age. Similarly, ART improved the CD4+ T cell counts in children (3-9 years) and young adults (10-20 years), but failed to do so in children below 2 years (Ohtsuki et al, 2000).

The key finding of this study was that DPPIV/CD26 levels corresponded to those of CD4+ T cell counts and inversely to HIV viral load, suggesting that DPPIV/CD26 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/CD26. 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-Rodríguez et al, 2002; Aso et al, 2012). Elevated DPPIV/CD26 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/CD26 levels, with these women having low levels of the virus showing that DPPIV/CD 26 could have some protective properties against the virus as depicted by these sex workers who do not seroconvert (Songok et al, 2010). Our findings also suggest that the administration of ART to HIV-treatment naïve patients boosted the levels of DPPIV/CD 26 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/CD 26. 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 Ryan, 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).

5. Conclusion

This study provided a strong rationale for further evaluation of the potential for use of DPPIV/CD26 as marker for ART treatment efficacy in children and young adults. This is based on the observation that DPPIV levels directly corresponded with CD4+ T cell counts but inversely correlated with HIV loads in children with ART use. Therefore, demonstrating the potential for use of DPPIV/CD 26 in developing diagnostic kits for ART monitoring and probably be used in future in the development of vaccine initiatives against the virus.

Conflict of Interest

The authors declare no conflict of interest.

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