Effect of Antiretroviral Drugs on Cd4 Cells and Viral Load in HIV Patients Attending Rift Valley Provincial General Hospital, Nakuru, Kenya

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Abstract

CD4 count and viral load are part of laboratory data, which give guidelines on commencement and subsequent monitoring of chemotherapy. Among the primary goals of Antiretroviral Therapy (ART) are optimal and durable suppression of viral load and the preservation and /or restoration of immunologic function. The objective of the current study was to assess the virological and immunological responses of the human immunodeficiency virus (HIV) -infected individuals with administration of ART and establish the relationship between CD4 count and viral load in the study population, assessing the effect of chemotherapy. The study was conducted on 80 individuals who attended the Voluntary Counseling and Testing (VCT) centre in the Rift Valley Provincial General Hospital, Nakuru, and who consented to the study. Parallel testing for HIV was performed using Determine and Uni-Gold HIV ½ test kits, and discordant results were confirmed by Enzyme Linked Immunosorbent Assay (ELISA). CD3, CD4 and CD8 counts were determined using Beckton Dickson (BD) FACScount while viral loads measured using Exavir load kit prior to commencement of ART regimens. Virologic and immunologic responses were determined by measuring CD4 counts and viral load at two weeks following commencement of chemotherapy and monthly for three months thereafter. Viral loads and CD4 counts for the study population were found to be highly inversely correlated ($r=0.948$, $p<0.001$).

Key words: Human Immunodeficiency Virus, Viral loads, CD4 counts, antiretroviral therapy.

Introduction

The Human Immunodeficiency Virus (HIV) type 1 and type 2 are etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions. HIV-1 is disseminated world wide while HIV-2 is principally found in the West African regions but subsequently found in some European and South American countries. Of the estimated 1.5 million people infected with HIV in Kenya, about 200,000 are in urgent need of antiretroviral (ARV) therapy (NASCOP, 2005). The government of Kenya is committed to increasing access to antiretroviral drugs as part of its wider "Declaration of Total War" on HIV/AIDS, and has therefore developed a plan for the rapid up-scaling of antiretroviral therapy (ART) to government hospitals in every province in Kenya (MOH,2004).

CD4 count is a good indicator of the immune status of the individual as it plays an important role in both humoral and cell mediated immune responses. In HIV infection, CD4 counts are used to determine the progress of disease and to predict the risk of developing HIV related complications (Mary, 2003). When individuals are infected with HIV for a long time, their CD4 count decreases indicating immunesuppression. The lower the CD4 count, the higher the vulnerability to opportunistic infections.

The plasma viral load has been used as a measure of HIV replication (Pallela *et al.,* 2003). During the period of primary infection in adults, viral load initially rises to high levels. Viral load assays are useful for indicating the prognosis of HIV infection, for indicating when asymptomatic patients should be treated, and also as a reference for subsequent monitoring of the virologic response to therapy (Paula *et al.,* 2001). CD4 count and viral load, being part of laboratory data, may give guidelines on initiation and monitoring of chemotherapy. Average normal CD4 counts are between 500-1600 cells/mm^3 of blood (Sheppard *et al.,* 2005) while normal CD4 counts in adults in Kenya ranges from 500-1800 cells/mm^3 (NASCOP, 2001). The primary goals of antiretroviral therapy are maximal and durable suppression of viral load, sustained rises in CD4 counts, preservation and / or restoration of immunologic function, improvement of quality of life and reduction of HIV-related morbidity and mortality (Carmona *et al.,* 2001). Antiretroviral (ARV) drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits. There are three classes of antiretroviral drugs that currently have been licensed: Reverse transcriptase inhibitors (RTIs) target construction of viral DNA by inhibiting activity of reverse transcriptase. There are two subtypes of RTIs with different mechanisms of action: nucleoside-analogue RTIs (NTRIs) are incorporated into the viral DNA leading to chain termination, while non-nucleoside – analogue RTIs (NNRTIs) distort the binding potential of the reverse transcriptase enzyme. Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions and Fusion inhibitors that block HIV from fusing with a cell's membrane to enter and infect it. In Kenya, the leading regimens to consider are: two nucleoside RTIs and protease inhibitor, two nucleoside RTIs and non-nucleoside RTI and three nucleoside RTIs (NASCOP, 2001). The individuals in the current study were given two nucleoside RTIs (Lamivudine+ Stavudine) and non-nucleaside RTI (Nevirapine).

Materials and Methods

Study Site and Study Population

The study was carried out between May – October, 2006 in the Rift Valley Provincial General Hospital (PGH), located in the Rift Valley Province of Kenya. The hospital is situated on the northern part of Nakuru town, 1.5 kilometres from the town centre. The hospital serves people from the entire Rift Valley Province. As a result of HIV campaigns, patients report to Counseling and Testing (VCT) Centre at the PGH for HIV testing. Other patients are referred for diagnostic HIV testing due to persistent and recurrent opportunistic infections. At the VCT centre, the patients undergo pre-test counseling, which includes being made to understand why it is important to undertake HIV testing, what it entails and what the results may imply. HIV testing is routinely carried out at the VCT centre. Patients who tested HIV positive were referred to the Centre for Comprehensive Care (CCC) for further counseling, and it was at the CCC that patients were advised to have their CD4 counts and viral load determined. Counseling at CCC included talking to patients to accept the results, the importance of living a positive life despite being HIV positive and on how they could improve their immune system by starting antiretroviral therapy (ART). Before the patients started ART, their viral load, CD3, CD4 and CD8 counts were determined after which they commenced ART.

Study Design

A cross sectional study design was used which involved selecting the subjects as they reported in VCT centers and obtaining information. Permission to carry out the study at the Nakuru Provincial General Hospital was approved by the hospital's administration.

Sampling and Sample Size Determination

Stratified sampling was done from the HIV positive individuals attending the VCT centre. They consented to participate in the study by signing a questionnaire. Participants of the study were randomly sampled by use of random numbers. A sample size of 80 patients was used. The patients who were sampled were referred to the CCC for further tests.

Screening for Human Immunodeficiency Virus Patients

A total of eighty individuals participated in the study after being sampled from a population of patients who had been confirmed to be HIV positive using two parallel rapid screening tests, (Determine HIV 1/2, USA and Trinity Biotech Uni-Gold, USA). Twelve males and sixty eight females of various ages participated in the study (Table 1). None of the female patients was pregnant.

Rapid Test Screening for Human Immunodeficiency Virus

Screening for HIV was carried out using two parallel tests simultaneously, the "Determine HIV 1/2" test (Abbot Laboratories, USA) and "Trinity Biotech Uni-Gold" test (Trinity Biotech, USA). Whole blood obtained by finger pricks was used. The determine HIV 1/2 test kits components were: Determine HIV 1/2 Test card, 2 cards (10 tests/card); HIV 1/2 recombinant antigen and synthetic peptide coated; 1 bottle (2.5 ml) Chase Buffer prepared in phosphate buffer. The Trinity Biotech Uni-Gold tests kits comprised of: 20 Test devices containing colloidal gold labelled with recombinant HIV proteins, recombinant HIV proteins as test Zone, and a control line, wash reagent (2ml), 20 disposable pipettes. When using the determine HIV 1/2 test kit, the protocol was carried out as outlined in the manufacture's manual (Piot *et al.*, 1988; Gurtler *et al.*, 1994). Briefly the tests were conducted as follows: to each labelled test card, droplets of whole blood produced by finger prick from an individual patient was applied to the sample pad. After blood was absorbed into the sample pad, one drop of chase buffer was then applied. The result was read after 15 minutes (up to 60 minutes). The test result was positive when two red bars appeared in both the control window and the patient window of the strip in the test card. The test result was negative when one red bar appeared in the control window of the strip and no red bar appeared in the patient window of the strip (Fig. 2).

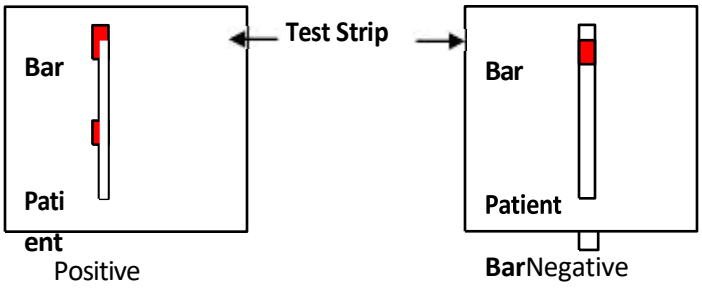


Figure 2:Bar Screening for HIV using Determine HIV1/2 Test

The Trinity Biotech Uni-Gold tests were carried out as outlined by the manufacturer (Feorino *et al.,* 1985; Atler *et al.,* 1987). Briefly, to each labelled test device, droplets of whole blood produced by finger prick from an individual patient were placed onto the device. Two drops of the wash reagent was added to the sample port. After 10-minute incubation time, the result was read. The test results were interpreted as follows: a line of any intensity forming in the test region of the test device, plus a line forming in the control region indicated a positive result while a line in the control region only indicated a negative test result (Fig. 3).

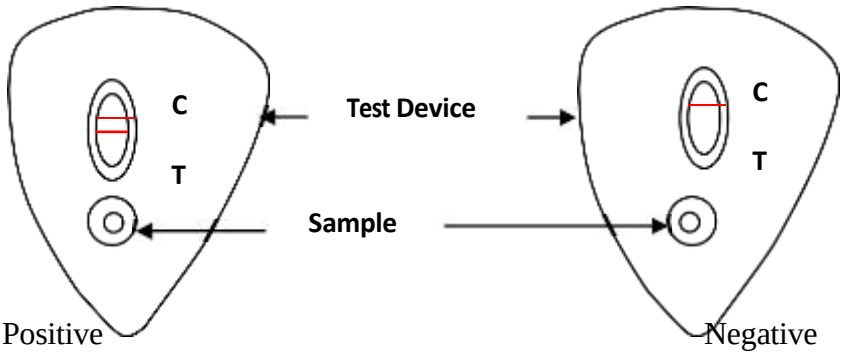


Figure 3: Screening for HIV using Trinity Biotech Uni-Gold test

Enzyme Linked Immunosorbent Assay (ELISA)

Discordant results from the two rapid tests were tested using Murex HIV 1.2.0 kit (Murex Biotech Limited, U.K). The components of the test kit were: Antigen coated wells; 96 microwells coated with HIV antigens; Sample diluent, Conjugate, Anti HIV 1 Positive Control Serum, Anti HIV 2 Positive Control Serum, Negative Control, Substrate diluent, Substrate concentrate and wash fluid. When using the Murex HIV 1.2.0 test kit, the protocol followed was as described by the manufacturer (Gains and Syndons, 1998). Briefly the test was carried out as follows: using test specimens and control sera, to precoated ELISA plates, 50µl of sample diluent was added followed by 50µl of serum sample in each well. After 30 minutes incubation period, unbound antibody was washed away, after which 50µl of HIV antigen conjugated to horseradish peroxidase was added to each well. The plate was incubated for 30 minutes and there after excess conjugate was washed away. Immediately after washing the plate, 100µl of substrate solution containing 3,3, 5,5' tetramethylbenzidine (TMB) and hydrogen peroxide was added to the wells. The plate was incubated for 30 minutes after which a purple colour developed in wells with bound conjugate, which was converted to an orange colour when the reaction was terminated with sulphuric acid and the optical density was read spectrophotometrically at 450nm. The amount of conjugate, and hence colour in the wells directly related to the amount of antibody to HIV in the sample. Guidelines to calculation of results were provided in the Murex HIV 1.2.0 test kit giving the mean absorbance and the cut off value as 0.280;

Results of the assay were considered negative when the samples gave an absorbance less than the cut off values, while the assay was considered positive when the samples gave an absorbance equal to or greater than the cut off value.

Data on Opportunistic Infection

Patients' data on opportunistic infection was recorded from their files as diagnosed before commencement of chemotherapy

CD4 Count Determination

CD4 counts were carried out using Beckton Dickson (BD) FACSCount system (BD Biosciences, USA) according to the manufacturers' protocol (David *et al.,* 2004). Beckton Dickson FACSCount is a complete system incorporating instrument, reagents, controls and software. It utilizes a direct two-colour immunoflourence method for enumerating absolute counts of CD3 lymphocytes, CD4 lymphocytes and CD8 lymphocytes. In addition the system generated a ratio of CD4 and CD8. The BD FACSCount reagent kit consisted of paired reagent sets containing a mixture of monoclonal antibody reagents conjugated to two fluorochromes and a known number of fluorochrome –intergrated polystyrene beads. The first tube in each pair contained CD4 and CD3 antibodies while the second contained CD8 and CD3. The kit also contained formaldehyde fixative. Briefly, the procedure was as follows: whole blood was collected in liquid EDTA; 50µl of whole blood was added to each tube, capped and vortexed. The samples were then acquired and run on the BD FACSCount instrument. The data was processed and reported on a sample print out sheet. CD4 counts were determined for all patients before and after commencement of chemotherapy, first at two weeks of therapy then monthly for three subsequent months.

Viral Load Determination

Viral load was determined using ExaVir Load kit (Cavidi Tech AB, Sweden) according to the protocol provided by the manufacturer (Malmstem e*t al*., 2003; Braun *et al.,* 2003). Briefly the ExaVir Load kit procedure was divided into two main parts: that is the separation and the reverse transcriptase (RT) – assay. In the separation part, the plasma was first treated to inactive cellular enzymes by adding 100µl of plasma treatment additive. 1ml of the sample was pipetted into each of the 32 plasma processing tubes placed in a sample box and incubated for 1 hour in the dark at room temperature. After the 1 hour incubation, 1.5ml of separation gel was added to each plasma processing tube and the sample box was placed on a moving table and incubated at room temperature for 90 minutes. The gel was meant for separating the virus particles from the plasma. After the 90-minute incubation, the gel was sucked dry in all the tubes using a vacuum pump, the gel was then washed four times using 250ml of gel wash buffer. The gel was sucked dry again and washed two times using 250ml of gel reconditioning buffer. 500µl of lysis buffer was added to each tube and the lysates were transferred to lysate collection tube. To obtain the Reverse Transcriptase (RT), the virion was then lysed and the lysate collected for further analysis. During the RT-assay the lysate was analyzed in an ELISA set up. The wells contained the RNA template bound to the bottom. A reaction mixture containing primer and an RT substrate was added to the plate together with the lysates. If the lysate contained any RT enzymes, the enzyme synthesized a DNA-strand. This product was detected with alkaline phosphate conjugate anti bromodeoxyribouridine antibody (α-BradU). The product was quantified by addition of a colorimetric Alkaline Phosphate (AP) substrate.

For comparison of results, in house HIV positive controls and in house negative controls were prepared. In house HIV positive controls: about 100ml of plasma prepared from a pool of EDTA blood from HIV positive patients was prepared by mixing samples with high and low HIV RT activity levels. When no plasma with determined RT amount was available, a pool was prepared that corresponded to 25,000 copies/ml. The material was aliquoted into 1.2ml portion and 1ml of one portion was used as a positive control. In house HIV negative control: about 100ml of a pool of plasma from healthy blood donors was prepared. The material was aliquoted into1.2ml portions and 1ml of one portion used as a negative control. When the AP substrate was added to the product, the plate was incubated in the dark at room temperature. The plate was read at an optical density of 405 (A_{405}) ten minutes after addition of the substrate. The plate was read a second time after two to three hours and a third time after five to six hours or the following day (16 to 24 hours) after addition of AP substrate. Calculation of the viral load values of the plasma samples was performed using the ExaVir Load Analyzer. Viral load determination was carried out in all patients before commencement of chemotherapy and thereafter, first at 2 weeks on therapy and monthly for three subsequent months while on chemotherapy.

Antiretroviral Therapy

Highly active antiretroviral therapy (HAART) was used. Highly active antiretroviral therapy is a combination of three or more antiretroviral drugs in the treatment of HIV infection. The drugs that were used were stavudine (D4T), lamivudine (3TC) and nevirapine (NVP). Doses for patients who were less than 60kg were D4T-30mg twice daily, 3TC-150mg twice daily and NVP-200mg twice daily. Doses for patients who were more than 60kg were D4T – 40mg twice daily, 3TC-150mg twice daily and NVP-200mg twice daily. Patients were advised to take NVP once daily for the first 2 weeks of treatment. They had to return for more drugs after two weeks. Highly active antiretroviral therapy was initiated in all patients with CD4 counts less than 200 cells/mm^3 irrespective of their viral load although 11 patients commenced treatment with counts more than 200 cells/mm^3 due to the severity of opportunistic infections.

Data Management.

CD4 counts and viral loads, as indications of patients' responses were analyzed using Chi-squire test for goodness of fit. The mean CD4 counts and mean viral loads for all the patients during chemotherapy were analyzed using Kruskal-Wallis test. The relationship between the total mean CD4 counts and the total mean viral loads during chemotherapy were analyzed using coefficient of correlation. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS version 18) software; generated data was then presented and interpreted using graphs and tables.

Results:

Twelve males and sixty eight females of various ages participated in the study (Table 1). Six patients (all females) out of eighty (7.5%) had discordant results by parallel testing for HIV. Four patients out of six (5.0%) were HIV positive with Determine HIV 1/2 test but negative when tested with Trinity Biotech Uni-Gold test. Two patients out of six (2.5%) were HIV

negative when tested with Determine HIV 1/2 test but positive when tested with Trinity Biotech Uni-Gold test.

Table 1: Gender and Age of the Study Population

Age in years	Males	Females	Total
Less than 21	0	5	5
21 – 25	2	11	13
26 – 30	3	18	21
31 – 35	2	23	25
36 – 40	3	6	9
More than 40	2	5	7
Total	12	68	80

The serum samples of the six discordant samples were tested for anti HIV antibody by Enzyme Linked Immunosorbent Assay (ELISA) using Murex HIV 1.2.0 Kit (Murex Biotech Limited, UK). All the six samples had absorbance values greater than the cut-off point (0.280) indicating that they were all HIV positive. The absorbance of the six samples were as follows; 0.342, 0.416, 0.402, 0.384, 0.301 and 0.408.

CD4 Levels and Clinical Manifestations

In all the patients included in this study, the highest CD4 count detected at the baseline was 220 cells/mm^3 of blood and the lowest was 8 cells/mm^3 of blood. CD4 counts were grouped into three categories depending on the symptoms and opportunistic infections present. Out of eighty patients, twenty seven (33.75%) had CD4 counts of less than 100 cells/mm^3 of blood at the baseline and a mean CD4 count of 54. The most common opportunistic infections by the patients with CD4 counts less than 100 cells/mm^3 of blood included prolonged weakness, chronic diarrhoea, tuberculosis, Kaposi's sarcoma, candidiasis of the oesophagus, Herpes simplex and pneumonia. Forty two patients (52.5%) had CD4 counts between 100-200 cells/mm^3 of blood at the baseline and a mean CD4 count of 151. They presented with persistent fever, pneumonia, tuberculosis and chronic diarrhoea. Eleven patients (13.75%) had CD4 counts of more than 200 cells/mm^3 of blood at baseline and a mean CD4 count 210. They presented with persistent generalized lymphodenopathy, Herpes zoster, recurrent upper respiratory infections and oral candidiasis.

The overall mean CD4 count before commencement of chemotherapy was 126 and all the patients were put on chemotherapy. After two weeks of chemotherapy the mean CD4 count increased to 148 (17.5% increase), after six weeks of chemotherapy the mean CD4 count increased to 209 (29.2% increase), after ten weeks of chemotherapy the mean CD4 count

increased to 252 (17.1% increase) and after fourteen weeks of chemotherapy the mean CD4 count increased to 278 (9.4% increase; Figure 4).

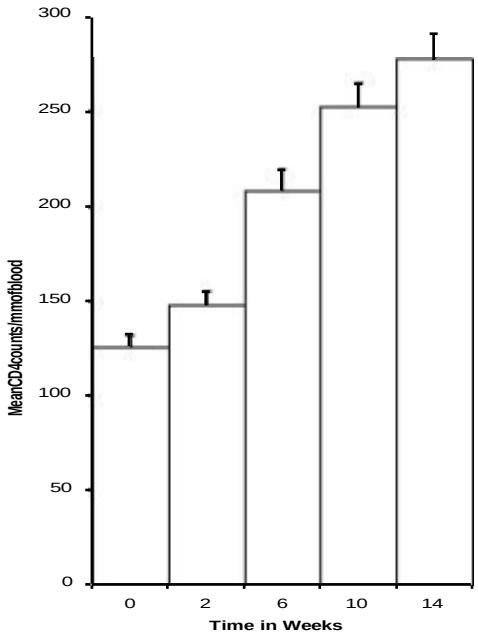


Figure 4: Mean CD4 count during chemotherapy

Viral Load and Clinical Manifestations

In all the eighty patients sampled, the highest viral load detected at the baseline was 1,900,000 copies/ml of plasma and the lowest was 100 copies/ml of plasma. Viral loads were grouped into three categories depending on symptoms and opportunistic infections present. Twenty-six (32.5%) patients had viral load of less than 50,000 copies/ml of plasma at the baseline and a mean viral load of 46,941. They presented with Herpes Zoster, oral candidiasis, recurrent upper respiratory infections and persistent generalized lymphodenopathy. Seven (8.75%) patients had viral load between 50,000 -100,000 copies/ml of plasma at the baseline and a mean viral load of 63,606. These patients presented with persistent fever, pneumonia, tuberculosis, chronic diarrhoea and oral candidiasis. Forty seven (58.75%) patients had viral load more than 100,000 copies/ml of plasma at the baseline and a mean viral load of 308,796. These patients presented with chronic weakness, chronic diarrhoea, Kaposi's sarcoma, candidiasis of the oesophagus, tuberculosis, Herpes simplex and pneumonia.

The overall mean viral load at the baseline for all the patients before commencement of chemotherapy was 419,343 and the patients were put on chemotherapy. After two weeks of chemotherapy the mean viral load decreased from 419,343 to 386,513 (7.83% decrease), after six weeks of chemotherapy the mean viral load decreased to 321,863 (16.73% decrease), after ten weeks of chemotherapy the mean viral load decreased to 289,077 (10.19% decrease) and after fourteen weeks of chemotherapy the mean viral load decreased to 265,537 (8.14% decrease; Figure 5).

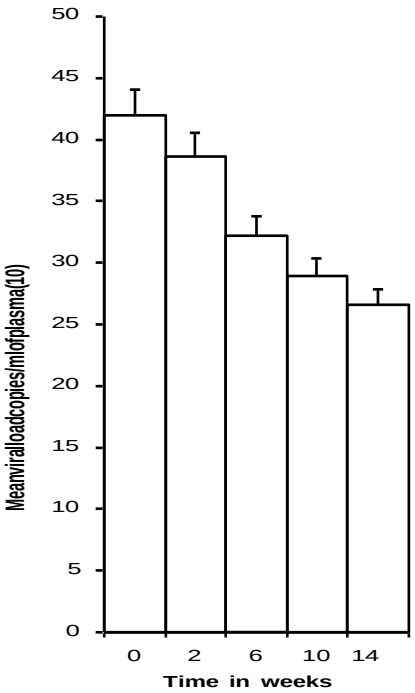


Figure 5: Mean viral load (RNA copies) during chemotherapy

Response to Chemotherapy in terms of CD4 Counts

Response to chemotherapy was monitored every two weeks for a period of fourteen weeks. Patients at different stages of infection were presented separately. After two weeks of chemotherapy, CD4 counts had increased in sixty-four (80%; Table 2), thirteen patients had decreased CD4 counts (16.3%) and there was no change in CD4 counts among three patients (3.7%; Table 2). Among the patients with CD4 counts less than 100 cells/mm3 of blood at the baseline, twenty one patients (77.8%) had increased CD4 counts two weeks after chemotherapy, five patients (18.5%) had decreased CD4 counts and there was no change in one patient (3.7%). Among the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline, thirty five patients (83.3%) had increased CD4 counts, six patients (14.3%) had decreased CD4 counts and there was no change in one patient (2.4%). For the patients with more than CD4 counts 200 cells/mm^3 of blood at the baseline, eight patients (72.7%) had increased CD4 counts in response to chemotherapy, two patients (18.2%) had decreased CD4 counts and there was no change in one patient (9.1%; Table 2).

Table 2: Effect of chemotherapy on CD4 count two weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts <100 cells	100 – 200 cells	> 200 cells	Total Patients
Increased	21 (77.8%)	35 (83.3%)	8 (72.7%)	64 (80%)
Decreased	5 (18.5%)	6 (14.3%)	2 (18.2%)	13 (16.3%)
No change	1 (3.7%)	1 (2.4%)	1 (9.1%)	3 (3.7%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After six weeks of chemotherapy, seventy four patients (92.5%) had increased CD4 counts while six patients (7.5%) had decreased CD4 counts (Table 3). Among the patients with CD4 counts of less than 100 cells/mm^3 of blood at the baseline, twenty five patients (92.6%) increased CD4

counts in response to chemotherapy while two patients (7.4%) decreased CD4 counts (Table 3). Among the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline, forty one patients (97.6%) had increased CD4 counts while one patient (2.4%) had decreased CD4 counts (Table 3). For the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline, eight patients (72.7%) had increased CD4 counts while three patients (27.3%) had decreased CD4 counts (Table 3).

Table 3: Effect of chemotherapy on CD4 count six weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	**Baseline CD4 Counts**			**Total**
	<100 cells	**100 – 200 cells**	**> 200 cells**	**Patients**
Increased	25 (92.6%)	41 (97.6%)	8 (72.7%)	74 (92.5%)
Decreased	2 (7.4%)	1 (2.4%)	3 (27.3%)	6 (7.5%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After ten weeks of chemotherapy, seventy four patients (92.5%) had increased CD4 counts in response to chemotherapy, four patients (5%) had decreased CD4 counts and there was no change in two patients (2.5%; Table 4). Among the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline, twenty six patients (96.3%) had increased CD4 counts and there was no change in one patient (3.7%; Table 4). For those with CD4 counts between 100 – 200 cells/mm^3 of blood at the baseline, 38 patients (90.5%) had increased CD4 counts, three patients (7.1%) had decreased CD4 counts and there was no change in one patient (2.4%). For the patients with more than 200 cells/mm ^{3} of blood at the baseline, ten patients (90.9%) had increased CD4 counts and one patient (9.1%) had decreased CD4 count (Table 4).

Table 4: Effect of chemotherapy on CD4 count ten weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	**Baseline CD4 Counts**			**Total**
	<100 cells	**100 – 200 cells**	**> 200 cells**	**Patients**
Increased	26 (96.3%)	38 (90.5%)	10 (90.9%)	74 (92.5%)
Decreased	- (%)	3 (7.1%)	1 (9.1%)	4 (5%)
No change	1 (3.7%)	1 (2.4%)	- (%)	2 (2.5%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After fourteen weeks of chemotherapy, seventy three patients (91.2%) had increased CD4 counts, four patients (5%) had decreased CD4 counts and there was no change in three patients (3.8%) from the previous count (Table 5). Among the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline, twenty six patients (96.3%) had increased CD4 counts in response to chemotherapy and there was no change in one patient (3.7%; Table 5). Among the patients with CD4 counts between 100-200 cell/mm^3 of blood at the baseline, thirty seven patients (88.1%) had increased CD4 counts while three patients (7.1%) had decreased CD4 counts and there was no change in two patients (4.8%; Tab.5). For the patients with CD4 counts

more than 200 cells/mm^3 of blood at baseline ten patients (90.9%) had increased CD4 counts in response to chemotherapy and one patient (9.1%) had decreased in CD4 counts (Tab.5).

Table 5: Effect of chemotherapy on CD4 count fourteen weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts			Total
	<100 cells	100 – 200 cells	> 200 cells	Patients
Increased	26 (96.3%)	37 (88.1%)	10 (90.9%)	73 (91.2%)
Decreased	-	3 (7.1%)	1 (9.1%)	4 (5%)
No change	1 (3.7%)	2 (4.8%)	-	3 (3.8%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

CD4 Profile during Chemotherapy

Response to chemotherapy by patients at different levels of HIV infection was compared fortnightly for a period of fourteen weeks. The mean CD4 count among patients with CD4 counts less than 100 cells/mm^3 of blood increased from 54 to 242 during the fourteen weeks of chemotherapy. The mean CD4 count among patients with CD4 counts 100-200 cells/mm^3 of blood increased from 151 to 335 while the mean CD4 count of patients with CD4 counts more than 200 cells/mm^3 of blood increased from 210 to 352 during the same period of chemotherapy.

When the response was compared during the first two weeks of treatment, patients with 100-200 cells/mm^3 were found to have a better response ($p<0.001$; $t=12.5032$) compared to patients with less than 100 cells/mm^3 and more than 200 cells/mm^3. After six weeks of treatment, patients with 100-200 cells/mm^3 were found to have a better response ($p<0.01$; $t=6.4687$) compared to patients with less than 100 cells/mm^3 and more than 200 cells/mm^3. After ten weeks of treatment, patients with less than 100 cells/mm^3 were found to have a better response ($p<0.01$; $t=4.889$) compared to patients with 100-200 cells/mm^3 and more than 200 cells/mm^3 and after fourteen weeks of treatment, patients with less than 100 cells/mm^3 were found to have a better response ($p<0.01$; $t=5.0053$) compared to patients with 100-200 cells/mm^3 and more than 200 cells/mm^3.

Response to chemotherapy between the categories over the entire fourteen weeks were compared by regression analyses. Patients with 100-200 cells/mm^3 were found to have significantly better response ($P<0.01$; $t = 19.7332$) than the patients with less than 100 cells/mm^3 and patients with more than 200cells/mm^3 of blood.

Response to Chemotherapy by Patients with Different Levels of Viral Load

After two weeks of chemotherapy, thirty three patients (41.3%) had decreased viral load, forty six patients (57.5%) had increased viral load and there was no change in one patient (1.2%; Table 8). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, thirteen patients (50%) had decreased viral load in response to chemotherapy, twelve patients (46.1%) had increased viral load and there was no change in one patient (3.9%). Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, four patients

(57.1%) had decreased viral load in response to chemotherapy and three patients (42.9%) had increased viral load. For the patients with viral load more than 100,000 copies/ml of plasma at the baseline category, sixteen patients (34%) had decreased viral load in response to chemotherapy and thirty one patients (66%) had increased viral load (Table 6).

Table 6: Effect of chemotherapy on viral load two weeks post chemotherapy

Effect of Chemotherapy on viral load	**Baseline Viral Load**			
	<50,000 copies/ml	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	13 (50%)	4 (57.1%)	16 (34%)	33 (41.3%)
Increased	12 (46.1%)	3 (42.9%)	31 (66%)	46 (57.5%)
No change	1 (3.9%)	-	-	1 (1.2%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After six weeks of chemotherapy, thirty four patients (42.5%) had decreased viral load while fourty six patients (57.5%) had increased viral load (Table 7). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, twelve patients (46%) had decreased viral load in response to chemotherapy and fourteen patients (54%) had increased viral load. Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, five patients (71%) had decreased viral load in response to chemotherapy and two patients (29%) had increased viral load. For the patients with more than 100,000 copies/ml of plasma at the baseline seventeen patients (36%) had decreased viral load in response to chemotherapy and thirty patients (64%) had increased viral load (Table 7).

Table 7: Effect of chemotherapy on viral load six weeks post chemotherapy

	Baseline Viral Load			
Effect of Chemotherapy on viral load	**<50,000 copies/ml**	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	12 (46%)	5 (71%)	17 (36%)	34 (42.5%)
Increased	14 (54%)	2 (29%)	30 (64%)	46 (57.5%)
Percentage of decrease (%)	46.15	71.43	36.17	-
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After ten weeks of chemotherapy, thirty eight patients (47.5%) had decreased viral load while fourty two patients (52.5%) had increased viral load (Table 10). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, thirteen patients (50%) had decreased viral load in response to chemotherapy and thirteen patients (50%) had increased viral load (Table 8). Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, three patients (42.9%) had decreased viral load in response to chemotherapy and four patients (57.1%) had increased viral load (Table 8). For the patients with viral load more than

100,000 copies/ml of plasma at the baseline, twenty two patients (46.9%) had decreased viral load in response to chemotherapy and twenty five patients (53.1%) had increased viral load (Table 8).

Table 8: Effect of chemotherapy on viral load ten weeks post chemotherapy

	Baseline Viral Load			
Effect of Chemotherapy on viral load	**<50,000 copies/ml**	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	13 (50%)	3 (42.9%)	22 (46.9%)	38 (47.5%)
Increased	13 (50%)	4 (57.1%)	25 (53.1%)	42 (52.5%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After fourteen weeks of chemotherapy, fourty seven patients (58.8%) had decreased viral load while thirty three patients (41.2%) had increased viral load (Table 9). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, sixteen patients (61.5%) had decreased viral load in response to chemotherapy and ten patients (38.5%) had increased viral load (Table 9). Among the patients with viral load 50,000-100,000 copies/ml at the baseline, four patients (57.1%) had decreased viral load in response to chemotherapy and three patients (42.9%) had increased viral load (Table 9). For the patients with viral load more than 100,000 copies/ml of plasma at the baseline, twenty seven patients (57.5%) had decreased viral load in response to chemotherapy and twenty patients (42.5%) had increased viral load (Table 9).

Table 9: Effect of chemotherapy on viral load fourteen weeks post chemotherapy

	Baseline Viral Load			
Effect of Chemotherapy on viral load	**<50,000 copies/ml**	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	16 (61.5%)	4 (57.1%)	27 (57.5%)	47 (58.8%)
Increased	10 (38.5%)	3 (42.9%)	20 (42.5%)	33 (41.2%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

Viral Load Profiles during Chemotherapy

Response to chemotherapy by patients at different levels of viral loads was compared fortnightly for a period of fourteen weeks. The mean viral load of patients with plasma viral load less than

50,000 copies/ml decreased from 46,940 to 26,985 during the fourteen weeks of chemotherapy. The mean viral load of patients with plasma viral load 50,000 -100,000 copies/ml decreased from 63,606 to 42,825 while the mean viral load of patients with plasma viral load more than 100,000 copies/ml category decreased from 308,796 to 195,728 during the same period of chemotherapy.

When the responses were compared during the first two weeks of treatment, patients with viral load 50,000-100,000 copies/ml were found to have a better response ($p<0.001$; $t = 48.4562$) compared to patients with less than 50,000 copies/ml and more than 100,000 copies/ml. After six weeks of treatment, patients with viral load 50,000 -100,000 copies/ml were found to have a better response ($p<0.001$; $t = 16.0503$) compared to patients with less than 50,000 copies/ml and more than 100,000 copies. After ten weeks of treatment, patients with less than 50,000 copies/ml category were found to have a better response ($p<0.001$; $t = 18.9713$) compared to patients with viral load 50,000-100,000 copies/ml and more than 100,000 copies/ml and after fourteen weeks of treatment, patients with less than 50,000 copies/ml were found to have a better response ($p<0.001$; $t = 23.0911$) compared to patients with 50,000 – 100,000 copies/ml and viral load more than 100,000 copies/ml.

Response to chemotherapy between the categories over the entire fourteen weeks were compared by regression analyses. Patients with more than 100,000 copies/ml category were found to have significantly better response (Figure 6; $P<0.001$; $t = 460.7554$) than the patients with 50,000-100,000 copies/ml and less than 50,000 copies/ml categories (Figure 6).

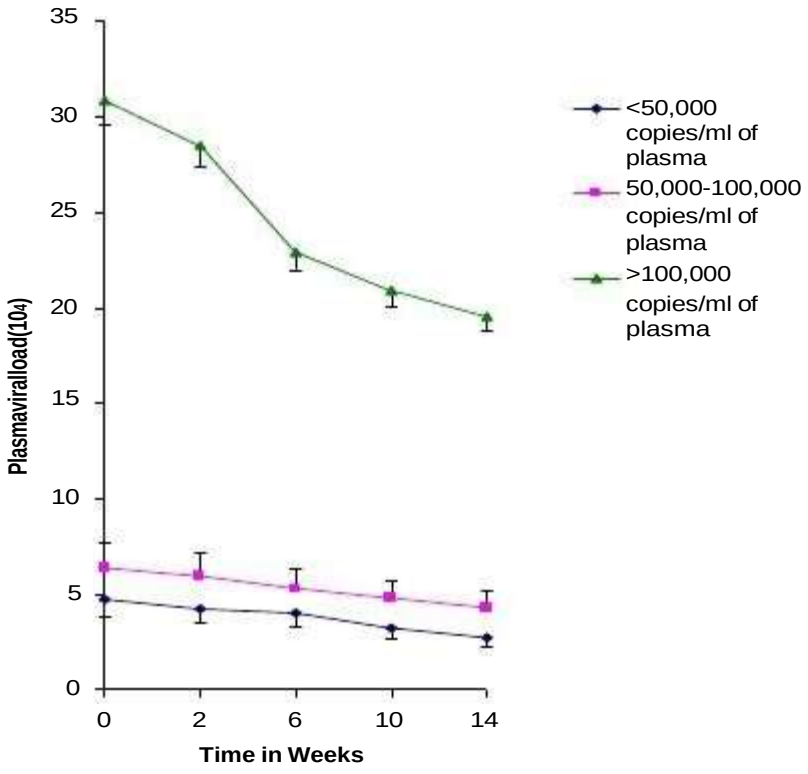


Figure 6: Viral load profile during chemotherapy. Patients categorized according to the level of viral load.

Comparison of Response in CD4 Counts and Viral Load during Chemotherapy

The mean CD4 counts and viral load of all the patients during chemotherapy were compared. Viral load and CD4 counts were found to be strongly inversely correlated (Figure 7; $P<0.001$; $r = -0.992$), that is, as CD4 counts increased, viral load decreased.

Further the relationship between response to CD4 counts and viral loads were compared for each of the categories of CD4 counts and viral loads. The mean CD4 count in the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline and the mean viral load in the patients with plasma viral load more than 100,000 copies/ml at the baseline were compared. The parameters were found to be strongly inversely correlated ($P<0.001$; $r = -0.983$).

Secondly the relationship between mean CD4 count in the patients with CD4 counts between 100-200 cells/mm3 of blood at the baseline and viral load in the patients with plasma viral load of 50,000-100,000 copies/ml at baseline were compared. The two parameters were found to have a very strong inverse correlation ($P < 0.001$; $r = -0.990$).

Finally the relationship between the mean CD4 count in the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline and viral load in the patients with plasma viral load less than 50,000 copies/ml at the baseline category were compared. The two parameters were observed to be strongly inversely correlated ($P<0.001$; $r = -0.969$).

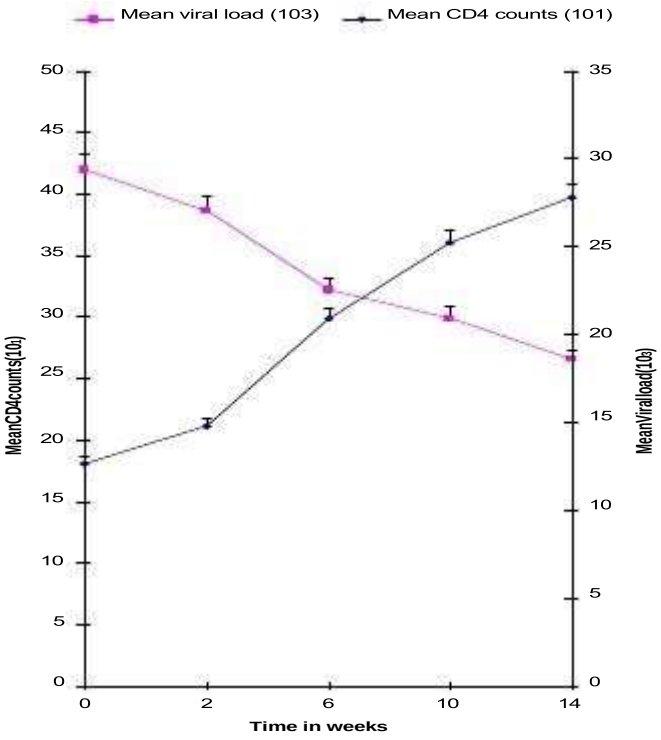


Figure 7: Mean CD4 counts and mean viral loads for all the patients during chemotherapy.

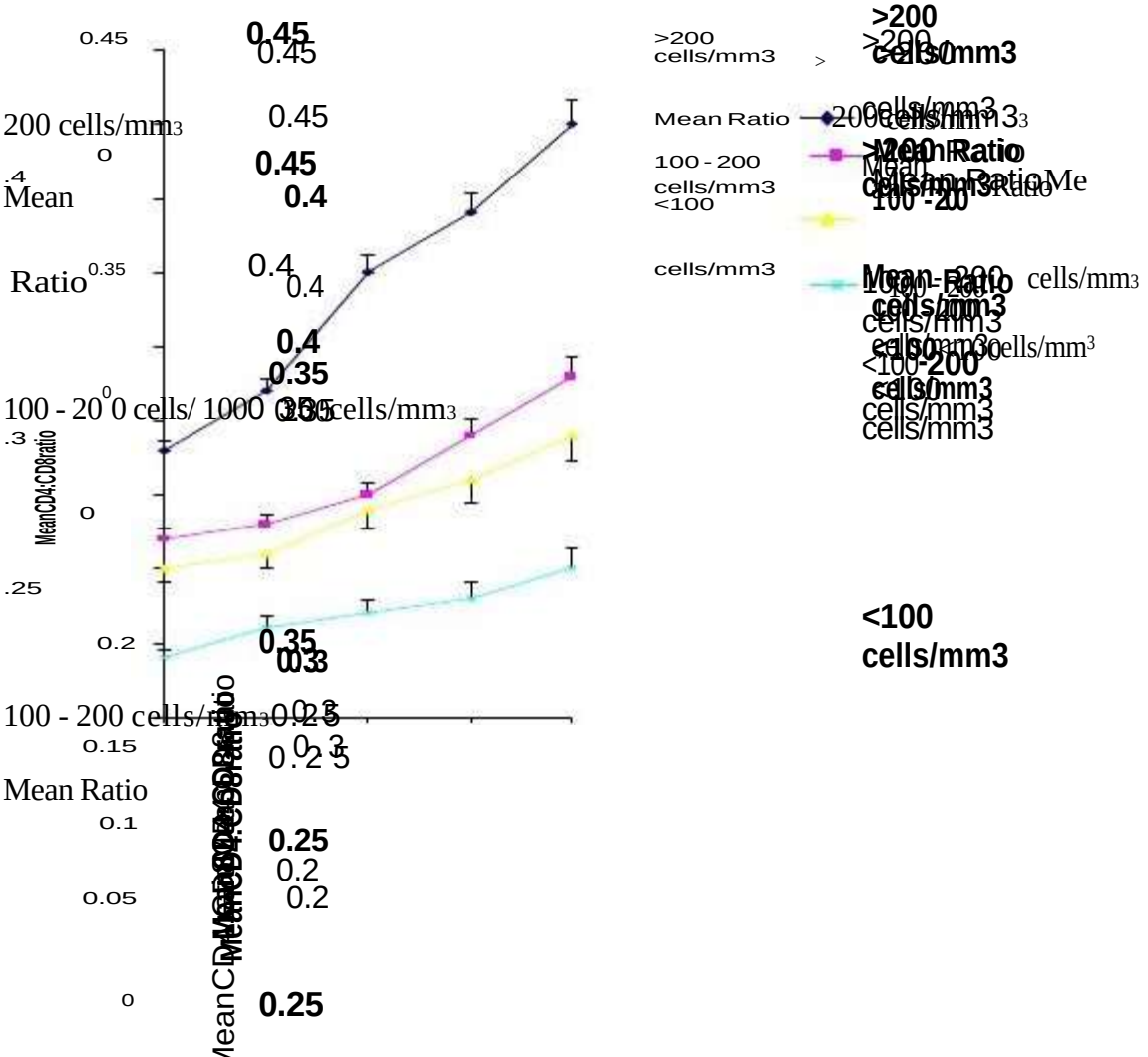
Effect of Chemotherapy on CD3 and CD8 counts

The mean CD3 count increased from 133 to 2078 and the mean CD8 count decreased from 1786 to 835 during the fourteen weeks of chemotherapy.

Effect of Chemotherapy on CD4 and CD8 ratio

The mean CD4:CD8 ratio for all the patients rose from 0.12 to 0.23 during fourteen weeks of chemotherapy (Figure 8). Changes in the CD4:CD8 ratio at different levels of CD4 counts were further examined. The mean CD4:CD8 ratio for the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline rose from 0.04 to 0.10 during the fourteen weeks of chemotherapy (Figure 8) while CD4:CD8 ratio for the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline rose from 0.10 to 0.19 during the fourteen weeks of chemotherapy (Figure 8). The CD4:CD8 ratio for the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline increased from 0.18 to 0.40 during the fourteen weeks of chemotherapy (Figure 8).

The change in ratio of CD4:CD8 among patients with different levels of CD4 counts during chemotherapy was compared. Patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline were found to have a significantly higher change (Figure 8; $P < 0.001$; $t = 39.91063$) in CD4:CD8 ratio than patients with less than 100 cells/mm^3 and patients with 100-200 cells/mm^3 of blood at the baseline categories.



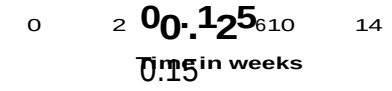


Figure 8: Effect of chemotherapy on CD4: CD8 ratio.

0.1
0.15
0.1

Discussion

In this study, six patients out of eighty had discordant results by pararell testing for HIV antibodies and a third test had to be performed for confirmation. In an earlier study, HIV screening using parallel testing for HIV antibodies recorded discordant results (Hellen, 2002) and a third test had to be used for confirmation. The serum samples of the discordant results in this study were tested for HIV antibody by enzyme linked immunosorbent assay (ELISA) and indicated that they were all HIV positive. This testing agrees with earlier tests carried out on discordant rapid tests which turned HIV positive using ELISA (Healthlink Worldwide, 1999). These results suggest that discordant results following rapid testing should not be concluded as outright negative.

In this study, all the patients had no prior treatment for HIV. They were given fixed dose combinations of stavudine and lamivudine to be taken twice daily and navirapine as an individual drug to be taken once daily. The antiretroviral drugs they received are among the recommended drug regimen to HIV patients by the Government of Kenya as the first line treatment for adults (MOH, 2004).

Overall during the entire period, the patients who started treatment with high viral loads (more than 100,000 copies/ml) had a significantly better response to treatment compared to the patients who started treatment with low viral loads (less than 100,000 copies/ml). This is in agreement with a study by Antony *et al*., (2002) who found better responses in patients who started treatment with high viral loads. As treatment progressed, there was improved health in all the patients. Reduced viral load was linked to improved health. It was observed that the patients who started treatment with viral loads over 100,000 copies/ml of plasma showed better health improvement over the entire period of the study compared to those who started treatment with viral loads below 100,000 copies/ml of plasma. This is in support of an earlier study where improved health was most noticeable in people who started treatment with high viral loads (http://www.atdn.org/simple/viral.html). This means that treatment with ARVs reduces viral load and improves the health of patients.

The mean CD4 count increased while the mean viral load decreased with chemotherapy, an indication of an improvement in immunologic function. Earlier studies have shown increases in mean CD4 counts and reduced viral loads with treatment. For example, one study by O'Brien (1996; http://www.aodsmuc.org/natap) showed that as the CD4 count increased, the plasma viral loads decreased during treatment. In previous studies it was reported that higher pre-treatment viral load and lower pre-treatment CD4 count were associated with greater increase in CD4 counts during the first three months of chemotherapy (Smith, 2004; Alatrakchi, 2005) resulting in the recovery of the immune function. Clinical benefits had been observed between eighth and fourteenth weeks of this study, and the clinicians agreed that the responses to antiretroviral therapy were evident. Generally, all the patients responded well to antiretroviral drugs although few had some delay in initial benefits, but prolonged treatment showed remarkable progress.

Conclusion

Progressive increases in CD4 count and reduction in viral load resulted in reconstitution of the immune system in most individuals in the study population, even in those with advanced disease

who started antiretroviral therapy at very low CD4 counts and very high viral loads. This substantially reduced the risk of clinical disease progression and death. Either CD4 counts or viral load could be used as an accurate measure of response to antiretroviral therapy.

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