THE EFFECTS OF ANTIRETROVIRAL DRUGS ON VIRAL LOAD IN HIV POSITIVE

PATIENTS ATTENDING NAKURU GENERAL HOSPITAL, KENYA

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ABSTRACT

The Human Immunodeficiency Virus (HIV) is the etiologic agent for Acquire Immunodeficiency Syndrome (AIDS). AIDS represents a global health crisis that threatens to overwhelm even the best health care delivery systems. Infection with HIV results in a progressive destruction of the CD4 lymphocytes, and subsequent development of HIV related opportunistic infections. The destruction of the T- cells is due mainly to active viral replication. A specific immune response to HIV occurs in HIV infected patients during primary infection and the strength of the primary immune response may be predictive of subsequent viral load in the body. Viral load are part of the laboratory data, which give guidelines on commencement, and subsequent monitoring of

chemotherapy. The primary goal of antiretroviral therapy is optimal and durable suppression of viral load, preservation and / or restoration of immunologic function, improvement of life and reduction of HIV related morbidity and mortality. An important aspect of antiretroviral treatment is accurate determination of when to commence and stop chemotherapy. Monitoring viral load is essential for managing therapy since there   is evidence that this important parameter may vary from individual to individual. The objective of the study was to monitor and assess the virological responses of HIV – infected individuals with administration of Antiretroviral drugs (ARVs) and establish the relationship between viral load and chemotherapy in the study population. The study was conducted between January and June, 2006, on people who were voluntarily attending Voluntary Counseling and Testing (VCT) centre in Nakuru General Hospital after getting consent from the hospital's administration. A cross sectional study design that involved selecting subjects and obtaining information was used to sample the study group and a total of 80 patients, 12 males and 68 females participated in the study. Screening for HIV was performed by parallel testing using Determine and UniGold HIV1/2 test kits. On testing HIV positive, the patients were referred to the Centre for Comprehensive Care (CCC), Nakuru, where they were referred for laboratory investigations. Their viral loads were measured using ExaVir Load Kit prior to commencement of antiretroviral regimens. Virologic responses to therapy were determined by measuring viral loads at two weeks interval on commencement of ARVs and monthly for three subsequent months thereafter. In all eighty patients, 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. The patients were categorized into three groups based on the viral loads at the baseline as: those with less than 50,000 copies/ml of plasma, those with between 50,000-100,000 copies/ml of plasma and those with more than 100,000 copies/ml of plasma.   The overall mean viral load before commencement of chemotherapy was 419,343 and after fourteen weeks of chemotherapy the mean viral load decreased to 265,537. Responses 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 (P<0.001; t = 460.7554) than the patients with 50,000-100,000 copies/ml and less than 50,000 copies/ml categories. Reduced viral loads were observed as early as two weeks after initiating chemotherapy, an indication that patients were responding to ARVs and achieving an

improvement in immunologic functions. Viral loads were found to be predictive of the benefits of chemotherapy. Data generated will be useful in improvement of HIV management strategies.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus in the family of lentiviruses and has been identified as the etiologic agent for acquired immunodeficiency syndrome (AIDS). Human immunodeficiency virus has been distinguished genetically and anti-genetically into two groups HIV-1 and HIV-2 (Mortimer, 2001). Human immunodeficiency virus 1 (HIV-1) contain 2 identical copies of a positive sense (that is mRNA) single-stranded RNA strand about 9,500 nucleotides long. These may be linked to each other to form a genomic RNA dimmer. The RNA dimmer is in turn associated with a basic nucleocapsid protein (Hooper, 2002). The ribonucleoprotein particle is encapsulated by a capsid made up of a capsid protein designated as p24.

The capsid environment also contains other viral protein such as integrase and reverse transcriptase. It also contains a wide variety of other macromolecules derived from the cell including tRNAlys3, which serves as a primer for reverse transcription. The capsid has an icosahedral structure (http://www.aidsnyc.org/natap). The capsid is in turn encapsulated by a layer of matrix protein designated as p17. The matrix protein is a continuous shell attached to the virus envelope. The HIV envelope is derived from the host cell plasma membrane and is acquired when the virus buds through the cell membrane (http://www.aidnyc.org/natap). A viral envelope contains the lipid and protein constituents of the membrane from which it is derived. In addition it also contains viral proteins often forming spikes or peplomers. The major HIV protein associated with the envelope is gpl20/41 (Peterlin, 1992). This functions as the viral antireceptor or attachment protein. Gp41 traverses the envelope and gp120 is present on the outer surface and is noncovalently attached to gp41. The precursor of gp120/41 (gpl60) is synthesized in the endoplasmic reticulum and is transported via the Golgi body to the cell surface (Janis, 1992). Human immunodeficiency virus type1 (HIV-1) and type 2 (HIV-2) are transmitted 'vertically' that is from mother to infant, and 'horizontally' through sexual intercourse and

through infected blood (Peter, 2001). The lymphocytes of a healthy carrier of HIV replicate and eliminate over one billion virions each day and the circulating virus load may exceed ten million virions per milliliter of blood (Mortimer, 2001). Transmission also depends on other factors including the concentration of HIV secreted into body fluids such as semen, secondary infections of the genital tract, the efficiency of epithelial barriers, the presence or absence of cells with receptors for HIV and the immune competence of the exposed person (Peter, 2001).

Progression of HIV from latent to patent infection is followed by a dramatic viral replication and maturation. Ongoing replication of HIV drives the disease process, causing progressive immunologic damage. Studies on the pathogenesis of HIV infection demonstrates that a continuous high-level of replication of HIV is present from the early stages of infection and at least 10^{10} particles are produced and destroyed each day (Antony, 2002). High rate of replication is found throughout the course of HIV infection. HIV replicates at the rate of around 10^8 to 10^{10} virus particles per day, probably giving rise daily to about 3×10^{-3} spontaneous changes (mutations) in its genetic sequence (Paula, 2001) . The ultimate size of a viral population containing a mutation is probably determined by three concurrent factors; the forward mutation frequency, the replicating capability of the mutated virus and the 'age' of the viral population containing the mutation that is how long ago this population was generated (Antony, 2002). With the on-going production of genetic variants of HIV there is then a continuous selection for the 'fittest' virus population (Paula, 2001). Sequencing studies reveal that no two AIDS patients carry the identical virus, furthermore, viral isolates taken from the same individual at different times also can differ substantially (http://www.aidsnyc.org/natap). The DNA sequence diversity seen in HIV is generated by its reverse transcriptase enzyme, which has been shown to be extremely error prone and thus give rise to numerous base substitutions, additions, and deletions. It has been estimated that between 5 and 10 errors are introduced into the HIV genome during each round of replication (Greene, 1992).

The plasma viral load has been used as a measure of HIV replication (Hammer, 2002). During the period of primary infection in adults, viral load initially rise to high levels. Coincident with the body's humoral and cell mediated immune response, viral load decline (National AIDS and STD Control Programme, 2001) Viral load assays are useful for indicating the prognosis of HIV

infection and for indicating when asymptomatic patients should be treated and also act as a reference for subsequent monitoring of the virologic response to therapy (Paula, 2001). Plasma viremia is a strong prognostic indicator in HIV infection (Mellors, 1996). Furthermore, reductions in plasma viremia achieved with antiretroviral therapy account for much of the clinical benefit associated with therapy (O'Brien, 1996).

MATERIALS AND METHODS

Study Area: The study was carried out in the Nakuru 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 kilometers from the town centre. The hospital serves people from the entire Rift Valley Province of Kenya which makes it an ideal centre for research. The authorized hospital capacity is 534 beds and 75 cots while the actual physical capacity is 453 beds and 46 cots.

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 viral loads 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).

Study Population: 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. 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. According to the VCT records, the monthly average attendance at the VCT was 100 patients. The patients who were sampled were referred to the CCC for further tests.

The sample size was determined using the formula, $n = \dfrac{Z^2qp^D}{d^2}$ as

Described by (Fisher, 1998). The target population was not known, so 0.5 was used for P. q =1-p; d = probability = 0.05; D = design effect = 1; n = sample size. Hence:

$N = \dfrac{2^2 \times 0.5 \times 0.5 \times 1}{0.05^2} = 400$

According to the records at the VCT, the attendance was less than 10,000 and so the following formulae was used (Fisher, 1998).

$nf = \dfrac{n}{1+n/N}$

nf = sample size estimate = 5
n = calculated sample size
N = monthly average attendance at the VCT.

The sample size was estimated as follows:

$nf = \dfrac{400}{1+ (400/100)} = 80$

A sample size of 80 patients was used.

Screening for Human Immunodeficiency Virus

A rapid test for 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). When using the determine HIV 1/2 test kit, the protocol was carried out as outlined in the manufacture's manual (Piot, 1988); (Gurtler, 1994); and the Trinity Biotech Uni-Gold tests were carried out as outlined by the manufacturer (Feorino, 1985); (Atler, 1987). Whole blood obtained by finger pricks was used.

Enzyme linked immunosorbent assay (ELIZA) was used to test discordant results from the two rapid tests were tested using Murex HIV 1.2.0 kit (Murex Biotech Limited, U.K). S. When using the Murex HIV 1.2.0 test kit, the protocol followed was as described by the manufacturer (Grains, 1998) . Guidelines for 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.

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

Viral Load Determination

Viral load was determined using ExaVir Load kit (Cavidi Tech AB, Sweden) according to the protocol provided by the manufacturer (Malmsten, 2003); (Braun, 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 lyses buffer was added to each tube and the lysates were transferred to lysates collection tube. To obtain the Reverse Transcriptase (RT), the virions 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.

Administration with 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 irrespective of their viral loads.

Data Analysis

Viral loads as indications of patients' responses were analyzed using Chi-squire test for goodness of fit. The mean viral loads for all the patients during chemotherapy were analyzed using Kruskal-Wallis test.

Results

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.

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

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. 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.

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 (Table 2). 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 (Table 2). 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 diarrhea and oral candidiasis (Table. 2). 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 diarrhea, and Kaposi's sarcoma, candidiasis of the esophagus, tuberculosis, Herpes simplex and pneumonia (Table.2).

Table 2: Viral load and corresponding clinical manifestations of patients

Viral load levels	Mean viral load	Number of patients	Opportunistic infections
Less than 50,000 copies/ml of plasma	46,941	26 (32.5%)	Persistent generalized lymphodenopathy Recurrent upper respiratory infections
50,000-100,000 copies/ml of plasma	63,606	7 (8.75%)	Oral candidiasis Herpes zoster Persistent/consistent fever Pneumonia Tuberculosis Chronic diarrhea Oral candidiasis
>100,000 copies/ml of plasma	308,796	47 (58.75%)	Chronic weakness Chronic diarrhea Kaposi's sarcoma Candidiasis of the esophagus Tuberculosis Herpes simplex 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 1).
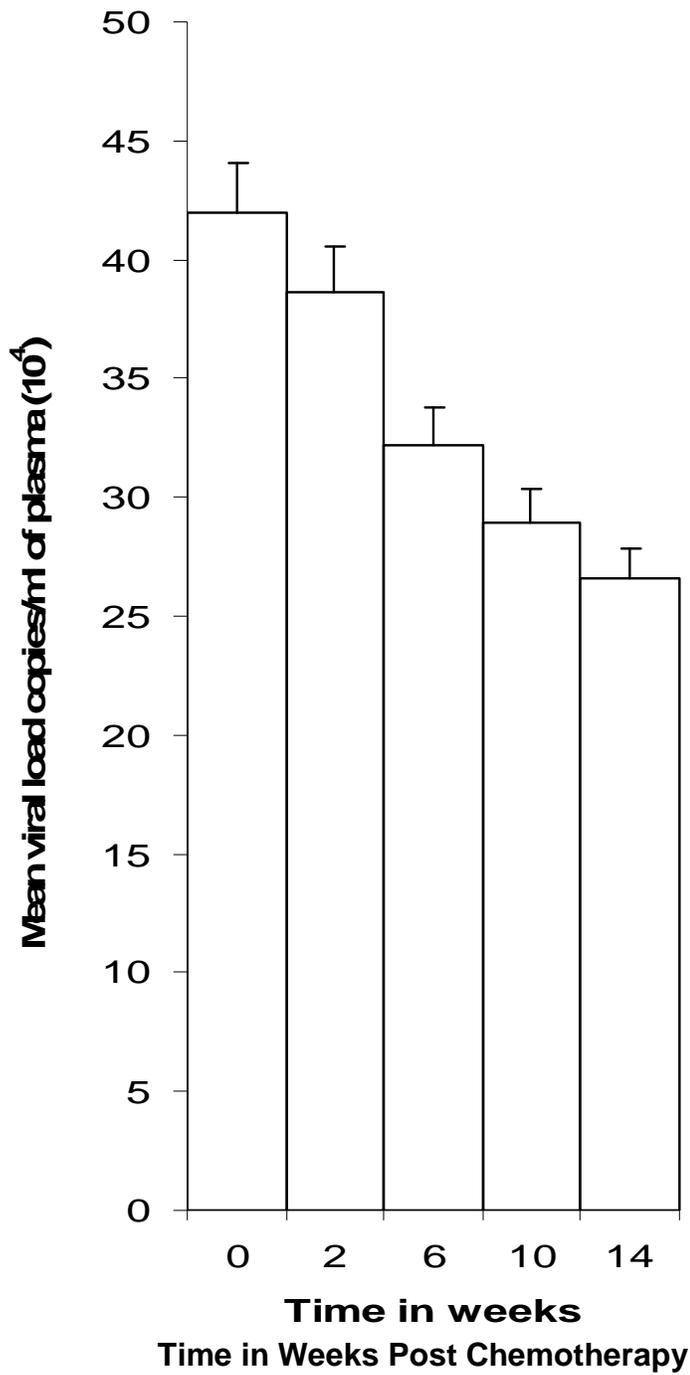
Figure 1: Mean viral load (RNA copies) during chemotherapy

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 3). 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 3).

Table 3: 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 forty six patients (57.5%) had increased viral load (Table.4). 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 4).

Table 4: Effect of chemotherapy on viral load six 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	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 forty two patients (52.5%) had increased viral load (Table 5). 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 5). 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 5). 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 5).

Table 5: Effect of chemotherapy on viral load ten 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%)	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, forty seven patients (58.8%) had decreased viral load while thirty three patients (41.2%) had increased viral load (Table 6). 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 6). 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 6). 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 6).

Table 6: 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 baseline plasma viral load less than 50,000copies/ml decreased from 46,940 to 26,985 during the fourteen weeks of chemotherapy. The mean viral load of patients with baseline plasma viral load 50,000 -100,000 copies/ml decreased from 63,606 to 42,825 while the mean viral load of patients with baseline

plasma viral load more than 100,000 copies/ml category decreased from 308,796 to 195,728 during the same period of chemotherapy (Figure 2).
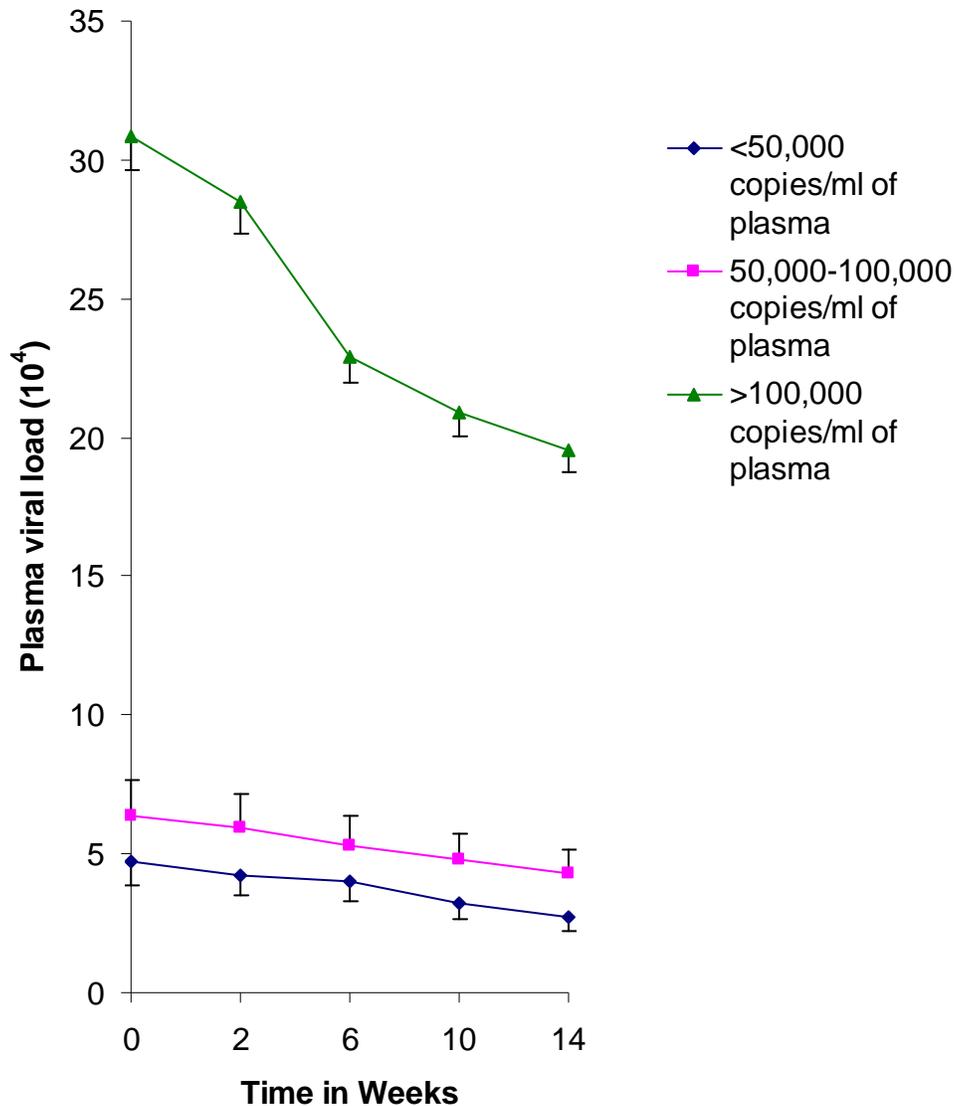

Figure 2: Viral load profile during chemotherapy. Patients categorized according to the level of viral load.

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.

Responses 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 2; P<0.001; t = 460.7554) than the patients with 50,000-100,000 copies/ml and less than 50,000 copies/ml categories.

DISCUSSION

Acquired immunodeficiency syndrome (AIDS) represents global health crises that threaten to overwhelm even the best health care delivery systems and it has emerged as the most terrifying epidemic of modern times. Over 20 million people have died since the first cases of AIDS were reported (Warren, 2005). The number of people living with HIV continues to grow and is currently about 40 million worldwide. Each day 14,000 men, women and children get infected; an epidemic that rages on (Khan, 2005). Although HIV and AIDS have now been identified in nearly all countries, the prevalence or scale of infection varies widely both between and within countries (Hellen, 2002).

Two rapid tests were used simultaneously (parallel testing) for detection of HIV antibodies to reduce the risk of error associated with rapid tests and also in support of the current recommendation on HIV screening based on earlier tests carried out where two rapid tests were found to be accurate in HIV diagnosis (Hellen C. , 2000). This is in line with recommendation that two rapid HIV tests should be conducted simultaneously to minimize the error (Worldwide., 1999). Two rapid tests are recommended for HIV screening because they have different sensitivity, specificity, and are based on different HIV antigens; their results are considered confirmatory for HIV if they agree.

In this study, six patients out of eighty had discordant results by pararel 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 J. , 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 (Worldwide., 1999). These results suggest that discordant results following rapid testing should not be concluded as outright negative.

Antiretroviral 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 (http://en.wikipedia.org/wiki/antiretroviral_drug). 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).

Viral load may vary from one individual to the other and from region to region. There are guidelines to consider when to initiate antiretroviral therapy (Paula, 2001), and the recommendations by the government of Kenya (NASCOP, 2001) were used when initiating ARVs to the individuals in this study. The individuals had various responses to ARVs which were attributed to their varied stages of HIV infection. Opportunistic infections and symptoms were common in individuals in this study especially those who had viral loads above 100,000 copies/ml of plasma. Antiretroviral drugs were used in addition to treatment for the opportunistic

infections in order to speed up recovery of the immune function. Newly presenting opportunistic infections were treated appropriately while maintaining the individuals on the antiretroviral regimens.

Laboratory tests indicated the degree to which the immune cells had been suppressed and the viral load accelerated which made individuals vulnerable to the opportunistic infections. This finding agrees with a study carried out in a South African Hospital, which showed that as HIV gradually weakened the immune system, signs and symptoms of different infections gradually became apparent (Hellen C. , 2000). In this study, the individuals whose viral loads were more than 100,000 copies/ml of plasma commonly presented with pneumonia of varying severity, Herpes simplex, tuberculosis, candidiasis of the esophagus, Kaposi's sarcoma, chronic diarrhea and chronic weakness. Those whose had viral loads between 50,000 -100,000 copies/ml of plasma commonly presented with chronic diarrhea, tuberculosis, pneumonia and consistent fever while individuals whose viral loads were   less than 50,000 copies/ml of plasma commonly presented with persistent generalized lymphodenopathy, oral candidiasis, recurrent upper respiratory infections and Herpes zoster. The symptoms and diseases corresponded broadly to levels of CD4 counts and viral loads suggesting varying degree of immunossuppression. This observation agrees with an earlier study which noted that when viral loads reached critical levels, the immune system was suppressed to such a degree that other infections gained entrance and the individuals were further weakened (Kassau A., 2001); 2001; (Tafteng Y. M., 2007).

Some patients increased viral load during chemotherapy an observation that also been reported (Hoffman, 1999); (Tafteng Y. M., 2007). In this study, the increase in viral load during treatment could have been attributed to persistent opportunistic infections. Opportunistic infections are extrinsic factors that may stimulate viral replication (Tafteng Y. M., 2007).
Viral load was observed to decrease with chemotherapy in this study. During the period reported here, the mean viral load in the study population reduced from 419,343 copies/ml of plasma pre-treatment to 265,537 copies/ml of plasma post-treatment. Higher percentage of patients who started treatment with low viral loads (less than 100,000 copies/ml according to this study) had higher viral suppression at weeks two and six while higher percentage of patients who started treatment with higher viral loads (more than 100,000 copies/ml) had higher viral suppression at

weeks ten and fourteen. This showed that patients who started treatment with low viral loads responded earlier and faster to ARVs but the viral suppression was not steady, while those who started treatment with high viral loads took longer to respond and maintain a steady viral suppression. This is in agreement with earlier studies carried out on viral suppression. One study (2002; http://www.thebody.com/content/treat/art/12234.html) noted that in the high viral load group, 100,000 copies/ml or more, patients took longer to achieve viral suppression. In another study, Wood (2005) reported that patients with more than 100,000 copies/ml were slower to suppress viral load when put on ARVs.

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, 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.

Clinical benefits had been observed between eighth and fourteenth weeks and the clinicians agreed that the responses to ARVs 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. Progressive 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 high viral loads. This substantially reduced the risk of clinical disease progression and death. Viral load could be used as an accurate measure of response to antiretroviral therapy.

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