THE EFFECTS OF ANTIRETROVIRAL DRUGS ON CD4 CELLS IN HIV POSITIVE PATIENTS ATTENDING NAKURU GENERAL HOSPITAL, KENYA

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ABSTRACT

The Human Immunodeficiency Virus (HIV) is the etiologic agent for Acquired Immunodeficiency Syndrome (AIDS). AIDS represents a global health crisis that threatens to overwhelm even the best health care delivery systems. The virus predominantly infects CD4 cells, which play an important role in the immune system. 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. CD4 T cell count and is part of the laboratory data, which give guidelines on commencement, and subsequent monitoring of chemotherapy. The collapse of the immune system in AIDS reflects the central role of CD4 T cells in both humoral and cell mediated responses and in the regulation of both responses. 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. CD4 T cell counts are essential for managing therapy and it is evident that these two important parameters vary from region to region. The objective of the study was to monitor and assess the immunological responses of HIV – infected individuals with administration of Antiretroviral drugs (ARVs) and more importantly the effect of chemotherapy was assessed. The study was conducted between January and June, 2006, on people who were voluntarily attending Voluntary Counseling and Testing 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, Nakuru, where CD4 counts were determined using BD FACScount prior to commencement of Antiretroviral regimens. Immunologic responses to therapy were determined by measuring CD4 counts at two weeks interval on commencement of ARVs and monthly for three subsequent months thereafter in all eighty patients, 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. The patients were categorized into three groups: those with less than 100 cells/mm^3 of blood, those with between 100-200 T cells/mm^3 of blood and those with more than 200 T cells/mm^3 of blood. The overall mean CD4 T cell counts before commencement of chemotherapy was 126 and after fourteen weeks of chemotherapy the mean CD4 count increased to 278. Patients had varied responses to chemotherapy. Increases in CD4 counts was observed as early as two weeks after initiating chemotherapy, an indication that

patients were responding to ARVs and achieving an improvement in immunologic functions. 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. Antiretroviral therapy was found to have effects on CD4 counts hence CD4 counts are predictive of the benefits of chemotherapy. Data generated will be useful in improvement of HIV management strategies.

Key words: Human Immunodeficiency Virus; CD4 T cell counts; Antiretroviral therapy; Immune responses.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a retrovirus. It was discovered by Barre' – Sinoussi, Montagnier and colleagues at the Institut Pasteur, Paris, in 1983 and given the name lymphadenopathy associated virus (LAV; Adler, 2001). In 1984, Popov Gallo and others described the development of cell lines permanently and productively infected with the virus (Mortimer and Loveday, 2001). Other virus isolates from patients with acquired immunodeficiency syndrome (AIDS) and AIDS – related disease in America, Europe and Central Africa have proved to be all the same virus, now referred to as HIV – 1 (Mortimer and Loveday, 2001). Around 1985 another human retrovirus different from HIV-1 was recognized in patients from West Africa. This virus, referred to as LAV-2 and later as HIV-2, is also associated with human AIDS and AIDS-related disease (Mortimer and Loveday, 2001). The human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV -2) are now recognized as the etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions.

Human immunodeficiency virus type 1 (HIV-1) is distributed worldwide while HIV-2 is principally found in the West Africa regions but has also been reported in some European and South American countries (Clavel, 1987). Infections with HIV are seen throughout the world and the major focus of the epidemic is in developing resource – poor countries (Clavel, 1987). The joint United Nations Programme on AIDS (UNAIDS) had estimated that by the end of 2000, there were 36.5 million people living with HIV/AIDS (34.7 million adults and 1.4 million children less than 15 years). The new infections during that year were 5.3 million, approximately 16,000 new infections per day. Currently, 95% of all infections occur in developing countries the major brunt of the epidemic being seen in sub-Saharan Africa and South and East Asia. At a family level it is estimated that by the end of 1999, the epidemic had left behind a cumulative total of 13.2 million AIDS orphans (Adler, 2001). The first AIDS case in Kenya was

diagnosed in 1984 and by 1995, 63,179 cases had been reported (MOH, 2002). Approximately 1.3 million adults and 100,000 children are currently infected with HIV in Kenya (NASCOP, 2005). Urban population has higher adult HIV prevalence (10%) than do rural population (6%; NASCOP, 2005).

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 and Mathew, 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 and Loveday, 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 and Mathew, 2001).

Human immunodeficiency virus (HIV) is immunosuppressive because it infects cells of the immune system and ultimately destroys them. T helper cells designated as CD4 cells are involved in humoral and cellular immune systems. Human immunodeficiency virus (HIV) primarily targets CD4 cells resulting in destruction of those infected by cytotoxic lymphocytes and subsequent elimination. Human immunodeficiency virus (HIV) carries ribonucleic acid (RNA) for genetic information, which has to be converted to deoxyribonucleic acid (DNA) both to establish persistent infection and to make virus replication possible. The conversion of RNA to DNA is performed by reverse transcriptase (RT) enzyme, which is encoded by the virus and packed in the virion. An active RT enzyme is therefore essential for virus replication. Thus infection of HIV can effectively be monitored by measuring the presence of RT activity (Malmsten *et al*., 2003).

The 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 HIV 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 immunosuppression (Peter and Mathew, 2001). Several studies have demonstrated that it's necessary to initiate antiretroviral therapy for patients with less than 200 CD4 cells/mm^3 of blood (Gulick *et al*., 1997; Stein *et al*., 1992; Rabound *et al*., 1996; NASCOP, 2001). However, it is important to note that the optimal time to initiate antiretroviral therapy among asymptomatic patients with CD4 counts more than 200 cells/mm^3 is still controversial (Mocroft *et al*., 1998; Palella *et al*., 1998; Vitinghoff *et al*., 1999).

Different recommendations have been given in different regions on when to start antiretroviral drugs. The British HIV Association (BHIVA) recommends that treatment should start when the CD4 cell count falls below 200 cells/mm^3 of blood, and may begin between 200 and 350 cells/mm^3 of blood, depending on the rate of CD4 decline, symptoms, patients' wishes and viral load (http://www.bhiva.org/guidlines.pdf). The United States department of health and human services (HHS) states that for people with 200 to 350 cells/mm^3 of blood antiretroviral therapy should generally be started. (http:www//.aidsinfo.nih.gov/guidelines/default-db2.aspid=50). An international AIDS society USA panel recommends starting treatment in patients with CD4 counts above 200 cells/mm^3 of blood in asymptomatic people with a CD4 count falling faster than 100 cells/mm^3 of blood yearly (Yeni, *et al.*, 2002). For poor and developing countries, the World Health Organization (WHO) advice treating anyone with AIDS, a CD4 count below 200 cells/mm^3 of blood or a total lymphocyte count below 1,200 cells/mm^3 of blood (http://www.who.int/docstore/hiv/scaling). In Kenya, recommendation on the decision to start therapy is made after considering the patient's acceptance or readiness and the probability of adherence. The strength of the recommendation is dependent on the prognosis as determined by clinical state, CD4 cell count and viral load (MOH, 2002). Antiretroviral therapy is recommended to start when a patient has CD4 count less than 200 cells/mm^3 of blood (NASCOP, 2001) and the government is committed to increasing access to antiretroviral drugs as part of it's wider "Declaration of Total War" on HIV/AIDS (MOH 2004), and has therefore developed a plan for the rapid upscaling of antiretroviral therapy (ART) to Government hospitals in every province in Kenya (NASCOP, 2005).Clinical benefit has been demonstrated in controlled trials only for patients with CD4 counts less than 200 cells/mm^3 of blood. However, most experts would offer therapy at a CD4 count less that 350 cells/mm^3 of blood and plasma HIV RNA of any value (MOH, 2002).

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. 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 CD4 counts 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, CD3, CD4 and CD8 T cell counts were determined after which they commenced ART.

Study Population: The individuals in this study sought voluntary counseling and testing (VCT) services at the Nakuru Provincial General Hospital. They needed HIV test to find out if HIV infection was the underlying cause of their medical problems and diagnostic testing to assist health staff to provide the best treatment. Prior to commencement of the study, an informed consent was obtained from the individuals before any medical tests, procedures or treatments were undertaken. The individuals were made to understand what the tests, procedures or treatment would involve, their purpose and how any findings would be used. After pre-testing counseling all individuals consented for an HIV test which would confirm diagnosis that was important for this study.

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. 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, $n = \frac{Z^2 qp^D}{d^2}$ as described by Fisher *et al.*,(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:

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

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

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

nf = sample size estimate = 5

n = calculated sample size

The sample size was estimated as follows:

$$nf = 1 + \frac{400}{100} = 80$$

A sample size of 80 patients was used.

Inclusion and Exclusion Criteria of the Study Subjects

There are many cases of HIV infection in Nakuru (Hospital records, 2005). Individuals in this study sought medical attention from Nakuru General Hospital due to varied medical problems presented by various signs and symptoms. They gave an informed consent for HIV screening and upon turning HIV positive, other tests were performed that included determination of CD4 counts. They were advised on the need to commence antiretroviral drugs and were made to understand the need to have their CD4 counts monitored to assess the effect of chemotherapy.

All members of the study group were all HIV positive and gave their signed consent to be included in the study. Those who did not consent were excluded from the study. The participants agreed to avail themselves for various tests whenever they were requested.

Blood Sample Collection, Screening for HIV and CD4 T cell Determination

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. 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 labeled test card, droplet 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. 1).

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. 2).

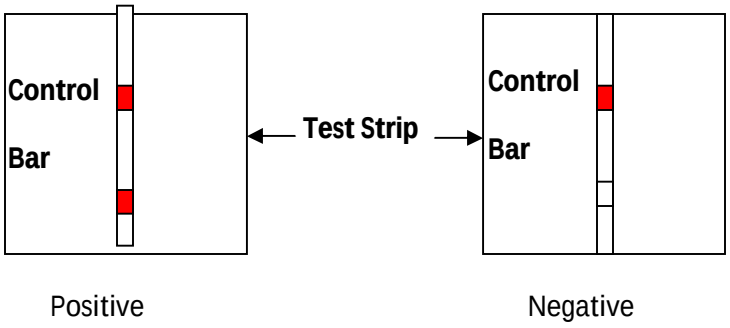

Figure 1: Screening for HIV using Determine HIV1/2 Test

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. 2).

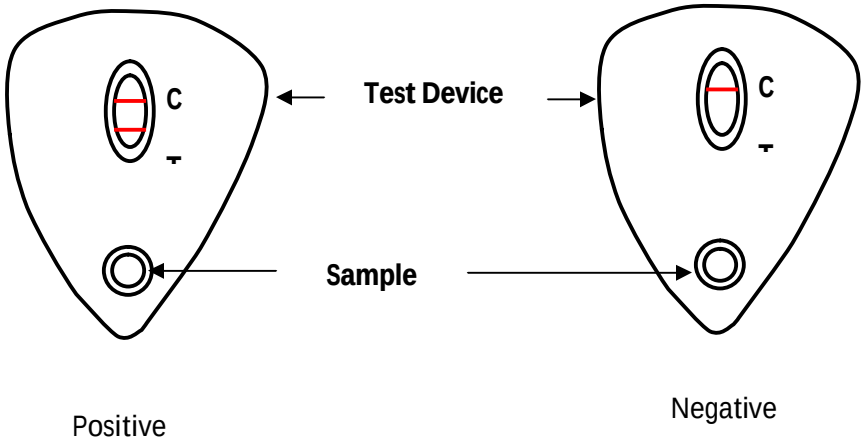

Figure 2: Screening for HIV using Trinity Biotech Uni-Gold test

Enzyme Linked Immunosorbent Assay (ELISA)

Discordant results from the two rapid tests were analzed by enzyme linked immunosorbent assay (ELIZA), using Murex HIV 1.2.0 kit (Murex Biotech Limited, U.K). When using the Murex HIV 1.2.0 test kit, the protocol followed was as described by the manufacturer (Gains and Syndons, 1998). 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.

Data Analysis

CD4 counts, viral loads as indications of patients' responses were analysed using Chi-squire test for goodness of fit. The mean CD4 counts and mean viral loads for all the patients during chemotherapy were analysed 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.

RESULTS

Description of the Study Subjects

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.

4.1 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 (Table 2). 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 (Table 2). 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 (Table 2). 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 (Table 2).

Table 2: CD4 Levels and corresponding clinical manifestations of patients

CD4 levels	Mean CD4 counts	Number of patients	Opportunistic infections
Less than 100 cells/mm^3 of blood	54	27 (33.75%)	Chronic weakness Chronic diarrhoea Kaposi's sarcoma Candidiasis of the oesophagus Tuberculosis Pneumonia
100 – 200 cells/mm^3 of blood	151	42 (52.5%)	Persistent/consistent fever Pneumonia Tuberculosis Chronic diarrhoea Oral candidiasis
>200 cells/mm^3 of blood	210	11 (13.75%)	Persistent generalized lymphodenopathy

Oral candidiasis

Recurrent upper

Respiratory infections

Herpes Zoster

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 3).

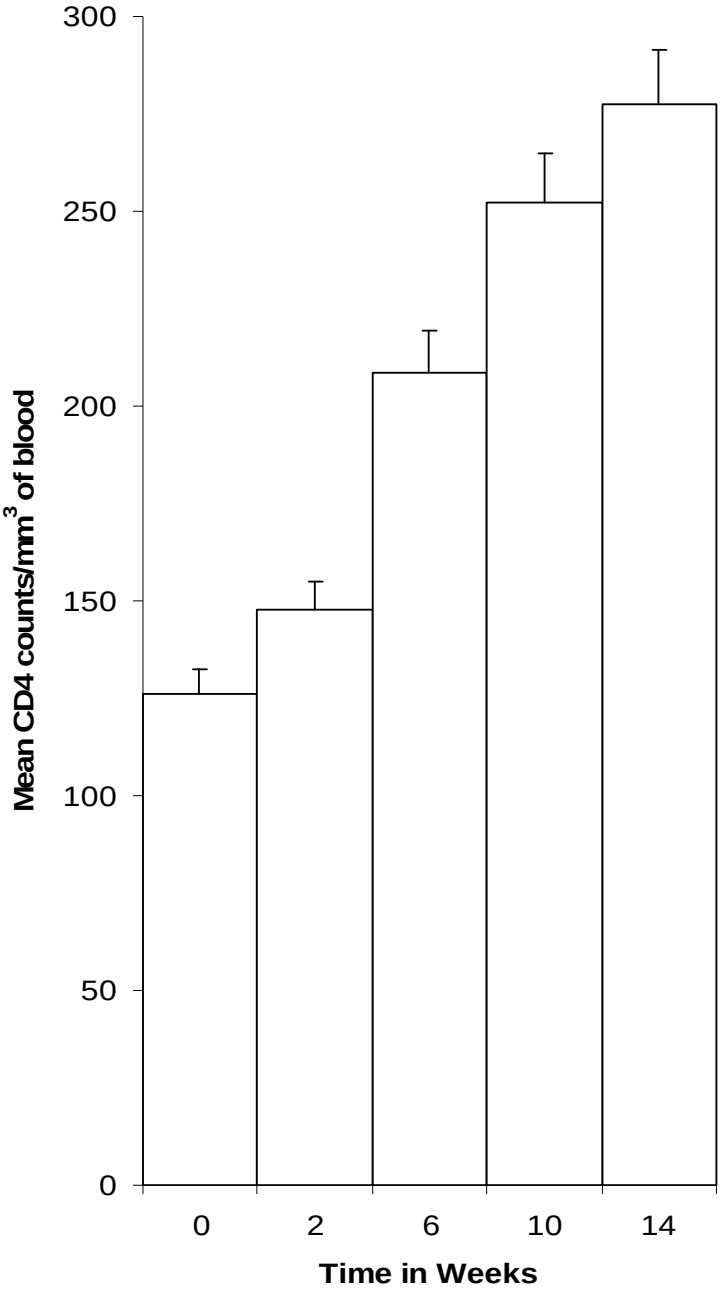

Figure 3: Mean CD4 count during chemotherapy

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, sixty four patients (80%) had increased CD4 counts, thirteen patients (16.3%) had

decreased CD4 counts, while there was no change among three patients (3.7%; Table 3). 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%; Table 3). 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%; Table 3). 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 3).

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

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts			Total
	<100 cells	**100 – 200 cells**	**> 200 cells**	**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 4). 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 4). Among the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline, fourty one patients (97.6%) had increased CD4 counts while one patient (2.4%) had decreased CD4 counts (Table 4). 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 4).

Table 4: 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 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 and there was no change in one patient (3.7%; Table 5). 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 5).

Table 5: 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 6). 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 6). 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%; Table 6). 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 (Table 6).

Table 6: 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 (Figure 3).

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 (Figure 4; $P<0.01$; $t = 19.7332$) than the patients with less than 100 cells/mm^3 and patients with more than 200cells/mm^3 of blood.

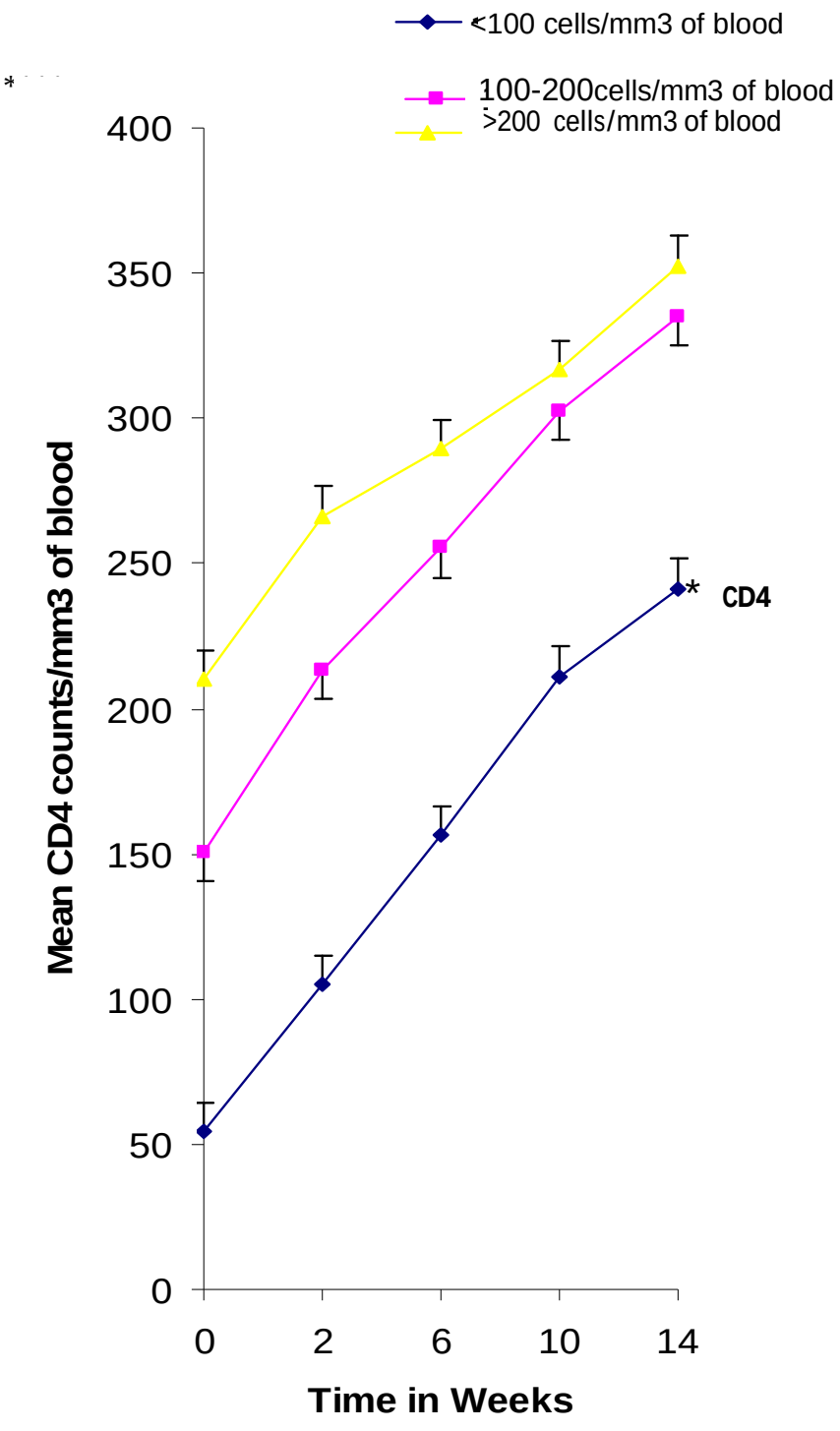

Figure 4: CD4 profile during chemotherapy. Patients categorized according to the level to the level of CD4 counts.

* Asterick means significantly better response in this category of patients than the patients in the other two categories. $P<0.01$; $t=19.7332$

DISCUSION

Acquired immunodeficiency syndrome (AIDS) represents a global health crisis that threatens 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 day14,000 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).

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).

CD4 counts may vary from one individual to the other and from region to region. There are guidelines to consider when to initiate antiretroviral therapy (Paula *et al.*, 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 CD4 count less than 200 cells/mm^3 of blood. 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.

Two rapid tests were used simultaneously (parallel testing) for detection of HIV antibodies to reduce the risk of error associated with rapid tests (Hellen, 2002) 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, 2000). This is in line with recommendation that two rapid HIV tests should be conducted simultaneously to minimize the error (Healthlink 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 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.

Individuals presented with various signs and symptoms that varied from person to person, but particular infections and a general pattern of disease emerged. The signs and symptoms of different infections were apparent in most individuals, in some cases including combination of two or more symptoms and disease, which they said had not responded to symptomatic treatment

over a few months. These diseases were diagnosed as either; bacterial, viral, fungal, protozoan or parasitic among other causes. The protozoal opportunistic infections included pneumocystis carinii pneumonia, viral infections included Herpes zoster and Herpes simplex, fungal infections included Candidiasis while bacterial opportunistic infections included tuberculosis. 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, 2002).

In this study, the individuals whose CD4 counts were less than 100 cells/mm^3 of blood commonly presented with pneumonia of varying severity, Herpes simplex, tuberculosis, candidiasis of the oesophagus, Kaposi's sarcoma, chronic diarrhea and chronic weakness. Those whose CD4 counts were between 100-200 cells/mm^3 of blood commonly presented with chronic diarrhoea, tuberculosis, pneumonia and consistent fever while individuals whose CD4 counts were more than 200 cells/mm^3 of blood 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 suggesting varying degree of immunossuppression. This observation agrees with an earlier study which noted that when CD4 cells reached critical levels, the immune system was suppressed to such a degree that other infections gained entrance and the individuals were further weakened (Kassau *et al*; 2001; Tafteng *et al*., 2007).

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). CD4 count increased with chemotherapy. The mean increase in CD4 count was 22cells/mm^3 at two weeks post treatment while the highest mean increase in CD4 counts was 61cells/mm^3 observed at six weeks post treatment. The increase in CD4 count at two weeks was an indication that the patients were responding to ARVs within a few days post commencement of therapy. This observation is in agreement with earlier work done by Tafteng

et al. (2007) which reported a CD4 count increase at 9 days post commencement of treatment. High percentage increases in CD4 count were observed at the tenth week in all patients, which could be attributed to improvement on immune system that was mounting a fight against opportunistic infections. In the majority of patients, most symptoms and opportunistic infections had subsided or disappeared at this point in time. Increases in CD4 count upon initiation of ARVs in patients have been extensively studied. For example, a study by Jansen (2006) showed increases in CD4 count during antiretroviral treatment. This reflects a general improvement of immune responses, which may have been induced by ARVs. Another study by Palella *et al.,* (2003) recorded increases in CD4 count with antiretroviral therapy. This is an indication that ARVs boosts the immune system by increasing the CD4 count.

The patients responded differently to antiretroviral treatment. Those who started treatment with baseline CD4 counts between 100-200 cells/mm^3 of blood had a significantly better response to treatment compared to those with low CD4 counts (less than 100 cells/mm^3) and high CD4 counts (more than 200 cells/mm^3). This observation agrees with an earlier study that showed a better response to treatment in patients with medium range CD4 count (180 cells/mm^3; Lawrence *et al.,* 2003). Lawrence's study was comparing treatment responses in individuals with CD4 count (180 cells/mm^3) midway between 398 cells/mm^3 from an earlier study (Ruiz *et al*., 2002) and with CD4 count of 30 cells/mm^3 from another study (Katlama *et al.,* 2003). This study does provide some data, which questions the prevailing wisdom that it is easier to achieve better immunological response if one begins ART at a higher CD4 count. These results are in agreement with other studies (http://www.thebody.com/content/treat/art2234.html) which showed no difference in CD4 count in groups that started ART with CD4 counts more than 200 cells/mm^3. This could mean an improved immune competence in these individuals as a result of better responses to treatments of opportunistic infections.

Some patients decreased CD4 count during chemotherapy. Decrease in CD4 count during treatment has been observed in other studies (Khan, 1992; Hoffman *et al*., 1999; Antoni *et al*., 2002; Tafteny *et al.,* 2007). In this study, the decrease in CD4 count during treatment could have been attributed to persistent opportunistic infections. Opportunistic infections are extrinsic factors that may stimulate viral replication (Tafteny *et al*., 2007). With an active viral replication, the rate of CD4 cells destruction might outweigh the rate of production of newer cells. This

could justify why this decrease was observed in some patients' CD4 counts even when they were put on ARVs.

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.

Improved ratio of CD4:CD8 was observed during the study. Normally the ratio of CD4:CD8 on the peripheral blood is about 2:1 (http://www.projinf.org/fs/bloodwork. html). The changes in ratio of CD4:CD8 in the patients who started treatment with higher CD4 counts were more significant compared to the patients who started treatment with lower CD4 counts. The change in ratio improved as treatment progressed and this is in agreement with an earlier study by Mary (2003) that showed improved ratio of CD4: CD8 during treatment. The mean CD3 count increased during the fourteen weeks of chemotherapy. This is in support with an earlier study conducted on HIV positive patients on ARVs which reported an increase in CD3 count during treatment (http://www.thebody.com/forum/aids/labs/archive/tcell/index. html). This means that there was control of damage to the immune system and the immune functions was being restored.

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 increases in CD4 count 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. This substantially reduced the risk of clinical disease progression and death. CD4 counts or viral load could be used as an accurate measure of response to antiretroviral therapy.

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