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

JANE NYAMBURA MUGWE
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Mugwe, Jane Nyambura
The effects of antiretroviral drugs
DECLARATION

This is my original work and has not been presented for a degree in any other University or any other award.

Signature ____________________________ Date 17/4/2009

Jane Nyambura Mugwe

SUPERVISORS' APPROVAL

We confirm that the candidate under our supervision carried out the work reported in this thesis, as the University supervisors.

Dr. Michael M. Gicheru

Department of Zoological Sciences

Kenyatta University, Nairobi, Kenya

Signature ____________________________ Date 17-4-2009

Prof. Zipporah Ng’ang’a

Department of Medical Laboratory Sciences

Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Signature ____________________________ Date 17th April 2009
DEDICATION

To my late mother for her encouragement, my daughter Terry for her motivation, my sister Eunice for her encouragement, Kamau's family, Patrick, Lucy, Latoya and my family for their moral support.
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4.6 Response to Chemotherapy by Patients with Different Levels of Viral Load 59
4.7 Viral Load Profiles during Chemotherapy 62
4.8 Comparison of Response in CD4 Counts and Viral Load during Chemotherapy 65
4.9 Effect of Chemotherapy on CD3 and CD8 counts 67
4.10 Effect of chemotherapy on CD4 and CD8 ratio 68

CHAPTER FIVE: DISCUSSION 70
5.1 Overview 70
5.2 HIV Screening Tests 72
5.3 Opportunistic Infections 73
5.4 Response to Chemotherapy 75

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS 80
6.1 Conclusions 80
6.2 Recommendations 80

REFERENCES 82

APPENDICES 90
APPENDIX I AUTHORIZATION LETTER 90
APPENDIX II QUESTIONNAIRE 91
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.1</td>
<td>Gender and Age of the Study Population</td>
<td>46</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>CD4 Levels and corresponding clinical manifestations of patients</td>
<td>48</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Viral load and corresponding clinical manifestations of patients</td>
<td>51</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Effect of chemotherapy on CD4 count two weeks post chemotherapy</td>
<td>54</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Effect of chemotherapy on CD4 count six weeks post chemotherapy</td>
<td>55</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Effect of chemotherapy on CD4 count ten weeks post chemotherapy</td>
<td>55</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Effect of chemotherapy on CD4 count fourteen weeks post chemotherapy</td>
<td>56</td>
</tr>
<tr>
<td>Table 4.8</td>
<td>Effect of chemotherapy on viral load two weeks post chemotherapy</td>
<td>59</td>
</tr>
<tr>
<td>Table 4.9</td>
<td>Effect of chemotherapy on viral load six weeks post chemotherapy</td>
<td>60</td>
</tr>
<tr>
<td>Table 4.10</td>
<td>Effect of chemotherapy on viral load ten weeks post chemotherapy</td>
<td>61</td>
</tr>
<tr>
<td>Table 4.11</td>
<td>Effect of chemotherapy on viral load fourteen weeks post chemotherapy</td>
<td></td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Schematic Representation of the Structure of HIV</td>
<td>9</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Mode of Action of Antiretroviral Drugs</td>
<td>26</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Screening for HIV using Determine HIV1/2 Test</td>
<td>39</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Screening for HIV using Trinity Biotech Uni-Gold test</td>
<td>40</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Mean CD4 count during chemotherapy</td>
<td>49</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Mean Viral load (RNA copies) during chemotherapy</td>
<td>52</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>CD4 profile during chemotherapy. Patients categorized according to the level of CD4 counts.</td>
<td>58</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Viral load profile during chemotherapy. Patients categorized according to the level of viral load.</td>
<td>64</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Mean CD4 counts and mean viral loads for all the patients during chemotherapy.</td>
<td>66</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Response to Chemotherapy in terms of CD3 and CD8 counts</td>
<td>67</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>Response to chemotherapy in terms of CD4: CD8 ratio</td>
<td>69</td>
</tr>
<tr>
<td>Acronym</td>
<td>Term</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------</td>
<td></td>
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<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
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<td>AP</td>
<td>Alkaline Phosphate</td>
<td></td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral Therapy</td>
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<td>ARV</td>
<td>Antiretroviral</td>
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</tr>
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<td>bDNA</td>
<td>branded Deoxyribonucleic Acid</td>
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<td>DNA</td>
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<td>EDTA</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
<td></td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescene Activated Cell Sorting</td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>Highly Active Antiretroviral Therapy</td>
<td></td>
</tr>
<tr>
<td>HHS</td>
<td>Health Human Services</td>
<td></td>
</tr>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>NASBA</td>
<td>Nucleic Acids Sequent Based Amplification</td>
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<td>NNRTIS</td>
<td>Non – nucleoside reverse transcriptase inhibitors</td>
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<td>NRTIS</td>
<td>Nucleoside or nucleotide reverse transcriptase inhibitors</td>
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<td>NVP</td>
<td>Nevirapine</td>
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<td>PIS</td>
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<td>QC – PCR</td>
<td>Quantitative Competition Polymerase Chain Reaction</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>RNA</td>
<td>Ribonucleic Acid</td>
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<td>RT</td>
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</tbody>
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RTIs - Reverse Transcriptase Inhibitors
RT-PCR - Reverse Transcriptase Polymerase Chain Reaction
STIs - Structured treatment interruptions
UNAIDS - United Nations Programme on AIDS
VCT - Voluntary Counseling and Testing
WHO - World Health Organization
The Human Immunodeficiency Virus (HIV) is the etiologic agent for Acquired Immunodeficiency Syndrome (AIDS). 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. It is important to monitor the viral load and CD4 Tcell count in order to determine the effectiveness of antiretroviral drugs. The objectives of this study was to monitor and assess the virological and immunological responses of HIV infected individuals with administration of Antiretroviral drugs (ARVs) and establish the relationship between CD4 count and viral load in the study population, more importantly the effect of chemotherapy was assessed. The study was conducted on people who attended voluntary counseling and testing centre in Nakuru General Hospital in the months of June to November, 2005 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 population 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 and viral load measured using ExaVir Load Kit prior to commencement of Antiretroviral regimens. Highly active antiretroviral therapy (HAART) was given to all the patients, fixed dose combinations of Stavudine and Lamivudine to be taken twice daily and Nevirapine to be taken once daily. Virologic and immunologic responses to therapy were determined by measuring CD4 counts and viral load 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 overall mean CD4 counts before commencement of chemotheraphy was 126 and after fourteen weeks of chemotherapy the mean CD4 count increased to 278. 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 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. Patients with CD4 counts less that 100 cells/mm$^3$ of blood at the baseline and patients with viral loads more than 100,000 copies/ml of plasma at the baseline had similar symptoms/opportunistic infections, while the patients with medium CD4 counts (100 – 200 cells/mm$^3$ of blood) and medium viral loads (between 500,000 – 100,000 copies/ml of plasma) at the baseline had similar symptoms/opportunistic infections. Patients had varied responses to chemotherapy. Increases in CD4 counts and viral load reduction 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. CD4 counts and viral loads were found to be strongly inversely correlated, $P<0.001; r = -0.992$. As the CD4 count increased, viral load decreased. Either CD4 counts or viral loads are predictive of the benefits of chemotherapy. Data generated will be useful in improvement of HIV management strategies.
CHAPTER ONE: INTRODUCTION

1.1 Background Information

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 type 1 (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/mm³ 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/mm³ is still controversial (Mocroft et al., 1998; Palella et al., 1998; Vitinghoff et al., 1999).

The plasma viral load has been used as a measure of HIV replication (Hammer, et al., 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 (NASCOP, 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 et al., 2001). Plasma viremia is a strong prognostic indicator in HIV infection (Mellors et al., 1996). Furthermore, reductions in plasma viremia achieved with antiretroviral therapy account for much of the clinical benefit associated with therapy (O’Brien et al., 1996).

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/guidelines.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 or with a viral load above 50,000 to 100,000 copies/ml of plasma (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, recommendations 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).
1.2 Study Justification

Monitoring of the viral load and CD4 counts is important in deciding when to start antiretroviral therapy (ART) in HIV infected individuals. It is also important in monitoring the response of chemotherapy and in deciding when it is safe to stop prophylaxis against opportunistic infections (http://www.aidsnyc.org/natap).

Nakuru region has high incidences of HIV (Hospital records, 2005) and there is a HAART programme in the Nakuru general hospital providing antiretroviral drugs to HIV patients. It is necessary to monitor the viral load and CD4 count on the study population who started treatment in order to establish the individuals’ responses to therapy in this region. In the course of HIV infection, CD4 cells and viral load have been known to vary from patient to patient and even from region to region and hence the responses should be monitored closely during chemotherapy. The influence of antiretroviral drugs (ARVs) on these two parameters may vary. In order to establish the efficacy of ARVs, there is need to establish response whenever antiretroviral drugs are being used. Among the primary goals of antiretroviral therapy are durable suppression of viral load, sustained rises in CD4 counts, restoration and /or preservation of immunologic function (Antony et al., 2002). According to International guidelines, therapy should be offered to all patients with CD4 count below 350 cells/mm3 and with viral load above 30,000 copies/ml (Paula et al., 2001). CD4 count and viral load should be measured after initiation of antiretroviral therapy to evaluate the initial effectiveness of therapy. If patients adhere to a regimen of potent antiretroviral therapy, viral load is expected to decrease by approximately 1.0 log_{10} by 2-8weeks (Antony et al., 2002). Highly active antiretroviral therapy leads to increases in the CD4 count, although individual responses are quite variable and in
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turn, continued viral load suppression is more likely among patients who achieve higher CD4 cell counts during therapy (Miller et al., 1999).

Like in many other drugs patients respond differently to antiretroviral drugs and it is necessary to determine CD4 counts and viral load before and after initiation of therapy to evaluate the initial effectiveness of therapy. Although highly active antiretroviral therapy (HAART) ought to lead to increases in CD4 counts and to continued viral load suppression, individual responses are quite variable, hence the need to determine these two parameters at each of the regions where HAART are being applied due to variation seen from region to region and between individuals. An earlier detection of change in CD4 count and viral load is required to optimize therapy and probably restore back the body’s immune system. Viral load and CD4 count are beneficial in monitoring of antiretroviral drug, allowing detection of decline of viral load from baseline to a desirable virologic end point and satisfactory increase in CD4 count while on therapy. For effective application of antiretroviral drugs and assessment of virologic and immunologic response to therapy, it is important to establish individuals’ responses by determining the viral load and CD4 count.

Nakuru district has high incidences of HIV as shown by the records of patients visiting the Centre for Comprehensive Care (CCC) of Nakuru Provincial General Hospital (PGH; Hospital records 2005). There is a HAART programme in the hospital that provides antiretroviral drugs to patients who have been referred from the Voluntary Counseling and Testing Centres (VCT), and there is need to evaluate the efficacy of the drugs in the region. There is also need to evaluate CD4 cells and viral load in accessing the response to antiretroviral drugs in the region.
1.2.2 Null Hypotheses

i) Antiretroviral drugs have no effect on CD4 count and viral load.

ii) There is no relationship between viral load and CD4 count on administration of antiretroviral drugs in HIV positive patients attending Nakuru Provincial General Hospital.

1.3 Objectives

1.3.1 General Objective

To determine the effects of antiretroviral drugs on CD4 counts and viral loads in HIV positive patients attending Nakuru Provincial General Hospital.

1.3.2 Specific Objectives

i) To monitor the effects of antiretroviral drugs on CD4 count in HIV positive patients attending Nakuru Provincial General Hospital.

ii) To monitor the effects of antiretroviral drugs on viral load in HIV positive patients attending Nakuru Provincial General Hospital.

iii) To establish the relationship between CD4 counts and viral load with the administration of antiretroviral drugs in HIV positive patients attending Nakuru Provincial General Hospital.
CHAPTER TWO: LITERATURE REVIEW

2.1 Human Immunodeficiency Virus (HIV)

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 antigenetically into two groups HIV-1 and HIV-2 (Mortimer and Loveday, 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 dimer. The RNA dimer is in turn associated with a basic nucleocapsid protein (p9/6; Hooper, 2000). The ribonucleoprotein particle is encapsulated by a capsid made up of a capsid protein designated as p24 (Figure 2.1).

Figure 2.1: Schematic Representation of the Structure of HIV (Source, http://www.aidsnyc.org/natap).
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 (Figure 2.1). 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 gp120/41 (Peterlin and Luciw, 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 (gp160) is synthesized in the endoplasmic reticulum and is transported via the golgi body to the cell surface (Janis, 1992).

2.2 Human Immunodeficiency Virus Infection

Upon infection with human immunodeficiency virus, several opportunistic infections will be presented during the primary HIV infection and at seroconversion. During the primary HIV infection there is 2-4 week period of intense viral replication before onset of an immune response and clinical illness. Clinical manifestations resolve as antibodies to virus become detectable in patient serum and then patients enter a stage of asymptomatic infection (Tindall and Cooper, 1991). At seroconversion illness is presented in several symptoms such as manifesting as a flu-like syndrome by which the general symptoms include acute onset of fever with or without night sweats,
myalgia is common, may be associated with muscle weakness, lethargy and malaise are frequent and often severe, may persist for several months, depressed mood, pharyngitis/sore throat, lymphadenopathy, arthralgia and anorexia (weight loss). Neurological symptoms at seroconversion may be presented by HIV readily being isolated from the cerebrospinal fluid and early infection of central nervous system frequently resulting in aseptic meningoencephalitis with symptoms of headache (Kinloch-De Loes et al., 1993). Other more unusual features include myelopathy, peripheral neuropathy, brachial neuritis, facial palsy and Guillain-Barre syndrome (Mary, 2003).

Gastrointestinal symptoms include mucocutaneous ulceration, pharyngeal edema is common, oral/oropharyngeal candidiasis, nausea/vomiting and diarrhea (Niu et al., 1993). Dermatological symptoms include erythematous, non-pruritic, maculopapular rash, roseola-like rash, diffuse urticaria, desquamation of palms and soles and alopecia (Schacker et al., 1996).

Several laboratory findings can be used to diagnose HIV infection during the primary infection and at seroconversion. During the first 1-2 weeks there is profound reduction in CD4 and CD8 lymphocyte counts with inversion of the CD4:CD8 ratio, the normal ratio is 2:1 (Mary, 2003). This is followed by a peripheral lymphocytosis consisting predominantly of CD8 cells and mild thrombocytopenia C-reactive protein level and erythrocyte sedimentation rate are frequently elevated, hemoglobin level usually remains stable and elevated serum alkaline phosphatase and transaminase levels are common (Pantaleo et al.; 1998). In the first 2-6 weeks of HIV infection, antibodies to HIV are detectable. HIV antigen (p24) may be detected in serum before
detecting antibodies; therefore, antigen testing is important in diagnosing seroconversion (Mary, 2003).

Many of the clinical features of HIV infection can be ascribed to the profound deficit that develops in infected individuals. The most obvious target of the virus is T lymphocytes carrying the surface molecule CD4, which has been shown to bind the envelope glycoprotein of HIV (gp120; Peter and Mathew, 2001). Entry of HIV into target cells involves two steps; binding of virions to CD4 receptors on target cells followed by fusion of the viral envelope with the plasma membrane of the target cells. The two glycoprotein that is gp120 and gp 41 that make up the surface projections on HIV play vital roles in these initial steps in HIV infections. Once inside a target cell, the viral genome is integrated into the host cell genome, forming a provirus, which may remain in a latent state or be activated and transcribed into viral protein (Janis, 1992). Virus entry also requires co-receptors, most of which are members of the seven transmembrane spanning G protein coupled receptor family (chemokine receptor; CXCR4, CCR2, CCR3, CCR5, CCR8, CCR9 and CX3CR1; Peter and Mathew, 2001). In the immune system these principally function as receptors for chemokines that orchestrate the migration, differentiation and function of leucocytes during immune responses. Two receptors, CCR5 and CXCR4 are particularly important. CCR5 (R5) is widely expressed on lymphocytes, macrophages, dendritic cells and cells of the rectal, vaginal and cervical mucosae. Virus strains able to infect primary macrophages (macrophage (M) or R5 tropic viruses) use CCR5 as a co-receptor. Only R5 strains are detected early after infection, while both R5 viruses and strains that infect T cells and use CXCR4 (X4) are found late in infection. It has been suggested that CCR5 strains are important for transmission of HIV while CXCR4
variants arise during the course of infection and may be responsible for T-cell loss and disease progression (Peter and Mathew, 2001).

After binding of HIV to its receptor (principally CD4), the viral envelope fuses with the target - cell plasma membrane. The fusion event appears to be induced by a hydrophobic region near the amino terminus of gp41 (Janis, 1992). Following fusion, the HIV nucleocapsid is internalized and the viral RNA is uncoated, establishing a productive infection (Peterlin and Luciw, 1992). Following HIV infection, a sequence of serological events occur that can be used to diagnose HIV infection and to predict the progression from latency to patent infection, culminating in AIDS diagnosis (Janis, 1992). Following infection, the virus appears to replicate actively and the viral core protein p24 can be detected by Enzyme-Linked Immunosorbent Assay (ELISA) or Radioimmunoassay (RIA; Mary, 2003). The p24 antigen is detectable in the serum only a few weeks following infection and then disappears as the antibody response (seroconversion) develops (Niu et al., 1993). Within a few weeks of seroconversion, IgG appears that is specific for many of the viral structural proteins including gp 160, gp 120, gp41, p24 and p17 (Peter and Mathew, 2001). As the antibody to p24 begins to decline, there is a corresponding increase in the appearance of p24 antigen in the serum. The decline in antibody to p24 and increase in p24 antigen are associated with the progression from latency to patent infection and have been used to clinically predict the onset of overt disease (Janis, 1992).

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 et al., 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 $3\times10^{-3}$ spontaneous changes (mutations) in its genetic sequence (Paula et al., 2001). The ultimate size of a viral population containing a mutation is probably determined by three concurrent factors; the forward mutation frequency, the replicative 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 et al. 2002). With the on-going production of genetic variants of HIV there is then a continuous selection for the 'fittest' virus population (Paula et al., 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).

### 2.3 Depletion of HIV Infected and Uninfected CD4 cells

As long as the HIV provirus in an infected CD4 cell remains in the latent state, no damage to the cell is evident. However, once the provirus is activated and new HIV virus begin to assemble and bud from the infected cell, extensive damage to the cell membrane can occur, leading to death of the cell. In addition, the humoral or cell mediated response generated against HIV may lead to destruction of HIV infected
CD4 cells. Those infected CD4 cells expressing gp120 and gp41 on their membrane can be killed by antibody + complement while those which express viral proteins associated with Class I MHC molecules can be killed by a CTL response against the altered self-cells (Gallo, 1992).

Several hypotheses have been fronted to account for depletion of uninfected CD4 cells. A human immunodeficiency virus infected CD4 cell can form a syncytium, by fusing with as many as 50 uninfected CD4 cells. These syncytia undergo lysis and death within 48 hours after their formation (Gallo, 1992). Some evidence suggests that soluble gp120 alone may interact with CD4 membrane molecules to induce cell fusion leading to syncytia formation. In one study a recombinant vaccinia virus containing the gp120 gene was able to cause syntia formation and subsequent cell death in a CD4 cell line in vitro. These findings suggest that the activated HIV-infected CD4 cells expressing gp120 with other, uninfected CD4 cells may lead to the progressive depletion of CD4 cells that is seen in AIDS patients (Weber and Weis, 1992). Other hypotheses have focused on the large quantities of soluble gp120 in the blood and lymph of AIDS patients. The noncovalent interaction of gp120 and gp41 is unstable, allowing large quantities of free gp120 to be shed into the surrounding fluid (Janis, 1992). Since gp120 has a high affinity for CD4, it can bind to CD4 molecules on normal, uninfected CD4 cells. Such binding might prevent antigen-mediated activation of T cells since CD4 plays a role both in stabilizing the interaction with an antigen – presenting cell and in transducing the activation signal (Schwarz, 1992). This has an effect on CD4 interaction with other cells in immune responses. Another possibility is that binding of soluble gp120 to CD4 membrane molecules may induce destruction of uninfected T cells by antibody + complement lysis or antibody
dependent cell-mediated cytotoxicity. The density of CD4 molecule is considerably higher on CD4 cells than on macrophages or other CD4-bearing cells (Janis 1992). This difference may explain why the depletion of CD4 cells can be extensive, while depletion of the other CD4-bearing cells is much less, on which the density of CD4 is too low to induce antibody-mediated complement activation or antibody-dependent cell-mediated cytotoxicity (Gallo, 1992).

The depletion of uninfected CD4 cells seen in AIDS normally would stimulate T-cell maturation within the thymus to restore the peripheral CD4 cell numbers. It has been suggested that free gp120 interferes with T-cell maturation within the thymus by binding to CD4 on thymocytes, thus interfering with positive selection of Class II MHC-restricted cells (Janis, 1992). It is therefore possible that the binding of soluble gp120 to CD4 might interfere with the maturation process. Destruction of mature CD4 cells in the periphery, coupled with a lack of replacement by developing thymocytes, could explain the progressive CD4 cell depletion seen in AIDS (Boehmer and Kisielow, 1992).

Although CD8 cells generally function as cytotoxic cells and CD4 cells as helper cells, the dichotomy is not absolute. Some CD8 cells secrete lymphokines and appear to function in a helper capacity and some CD4 cells have been shown to have cytotoxic activity (Janis, 1992). Experiments have suggested that in AIDS patients a CD4 cell population with cytotoxic activity may play a role in the destruction of uninfected CD4 cells (Weber and Weis, 1992). Another mechanism to account for depletion of uninfected CD4 cells in AIDS patients has been proposed. It has been suggested that binding of gp120 to CD4 may result in an inappropriate
transmembrane activating signal, transduced by a particular tyrosine kinase known to
be associated with the cytoplasmic tail of CD4 (Gallo, 1992). According to this
hypothesis, such inappropriate T-cell activation may prevent memory-cell formation,
leading to eventual exhaustion of T- memory cells and a gradual decrease of CD4
cells (Peterlin and Luciw, 1992). This hypothesis can account for some of the unusual
findings seen in AIDS patients, such as the generalized level of non-specific immune
activation and the ensuing polyclonal B - cell activation, the spontaneous lymphocyte
proliferation, and the increased autoimmune manifestation (Janis, 1992).

CD4 cells have been termed ‘the leader of the immunological orchestra’ because of
their central role in the immune response (Peter and Mathew, 2001). When these cells
are stimulated by contact with an antigen they respond by cell division and the
production of lymphokines, such as interferons, interleukins, tumour necrosis factor
and the chemoattractant chemokines (Peter and Mathew, 2001). Lymphokines act as
local hormones controlling the growth, maturation and behaviour of other
lymphocytes, particularly the cytotoxic/suppressor (CD8) T-cells and antibody –
producing B- lymphocytes. Lymphokines also affect the maturation and function of
monocytes, tissue macrophages and dendritic cells (Peter and Mathew, 2001). Within
days or weeks after HIV infection there may be a transient fall in CD4 lymphocyte
numbers and a more sustained rise in the number of CD8 cytotoxic/suppressor cells.
Among the CD8 cells, expanded oligoclonal populations are frequently seen and some
of those express a specific response to HIV (Mary, 2003).
Elevated CD8 counts are a general reflection of HIV infection as CD8 cells are the major cells associated with fighting the infection. CD3 is a marker for all T lymphocytes which include T helper cells (CD3 and CD4) and T suppressor or cytotoxic cells (CD3 and CD8; http://www.thebody.com/Farum/AIDS/Labs/Archive/Tecell/Q158227.htm).

2.4 Monitoring Human Immunodeficiency Virus Infection

Individuals may remain healthy for long periods, but a hallmark of disease progression, often prior to development of clinical symptoms, is a fall in the number of CD4 cells (Peter and Mathew, 2001). CD4 count is a key measure of the strength of the immune system in HIV positive persons, and is an important part of monitoring HIV infection. It is evident that a progressive decline in CD4 cells reflect disease progression and decreased life expectancy even in the absence of symptoms (Peter and Mathew, 2001), and their destruction accounts at least in part to the immunosuppressive effect of the virus. The spectrum of the immune dysfunction in AIDS is characterized by depletion of the CD4 cells, CD3 cells, decreased anti-HIV antibodies and an increase in activated and unresponsive CD8 cells (Mary, 2003).

Early in the disease, cytotoxic lymphocyte (CTL) activity against HIV-infected cells can be detected, but as the disease progresses, the CTL activity appears to decline (Lane and Fauci, 1985).

In addition to direct killing, CTL may contribute to protection by producing several chemokines and cells antiviral factor (CAF), which strongly inhibit viral replication in CD4 cells. Within days or weeks after infection there may be a transient fall in CD4 lymphocytes numbers and a more sustained rise in the numbers of CD8
cytotoxic/suppressor cells (Peter and Mathew, 2001). On average normal CD4 counts are between 500-1600 cells/mm$^3$ (Sheppard et al., 2005) while CD4 in adults in Kenya ranges from 500-1800 cells/mm$^3$ (NASCOP, 2001). Normally the ratio of CD4 to CD8 T cells in the peripheral blood is about 2:1, but in AIDS patients the ratio is reversed, becoming less than 1:2 and sometimes reaching levels as low as 1:5 (http://www.projin£org/lfs/bloodwork.html). Normal CD8 counts are between 375 and 1100 cells/mm$^3$ http://www.partes.de/applications/CyFlow Guav. pdf.)

Biopsy of the lymph nodes in patients with persistent generalized lymphadenopathy shows many enlarged follicles, often infiltrated by CD8 cells, with depletion of CD4 cells (Peter and Mathew, 2001). Even in clinically silent HIV infection, lymph nodes are the site of remarkably active HIV replication (Peter and Mathew, 2001). Viral burden in peripheral blood can be determined by using quantitative HIV RNA assays. During the period of primary infection in adults, HIV RNA copies initially rise to high levels. Coincident with the body’s humoral and cell-mediated immune response, RNA levels decline (http://www.aidsnyc.org/natap). Several studies conducted among adults have indicated that infected persons with lower HIV copy number have slower progression and improved survival compared with those with higher HIV RNA copy numbers (MOH, 2004).

CD4 cells counts for adults are used to decide when to start treatment, to monitor the response to treatment and to decide when it is safe to stop prophylaxis against opportunistic infections (Barcellos, 2002). The standard method for enumerating CD4 cells uses flow cytometer, a machine in which the cells of interest in a sample of blood are tagged with fluorescent monoclonal antibodies and passed in a single-cell
column in front of laser light. When the light illuminates a cell, the light is scattered in a pattern that can be read by a photosensor to indicate the cells size. Simultaneously, when the laser light strikes an antibody, it glows brightly and the cell is counted by a sensor attached to a microscope. A computer calculates the number of CD4 cells by analyzing the size of the cell and which of the antibodies it has been tagged with. The overall process is called fluorescence – activated cell sorting (FACS). Enumeration of CD4 counts are expensive because FACS machines are expensive, the antibodies are expensive and trained persons are necessary to perform the tests and maintain the equipment.

Cyflow (Patec Incorporation, USA) is a portable flow cytometry system that is less expensive and more robust than conventional FACS systems, uses less expensive reagents and is able to produce an absolute CD4 count without additional instruments (Teav, 2004). Total lymphocyte counts (TLC) can be used as a surrogate for CD4 counts, when combined with clinical evaluation (Thakar, 2004; Diagbouga, 2002). While the total lymphocyte count correlates relatively poorly with CD4 count, in combination with clinical staging it is useful marker of prognosis (Hammer et al., 2002). There are alternative systems, which use microscopes to count CD4 cells in the blood and include Dynaheads (Dynal Biotech, USA) and Coulter Cytospheres (Beckman coulter). The Dynabead (Magnetic beads) method uses two different sets of beads. One type (CD14 beads) binds to monocytes and is used to remove them. The second type (CD4 beads) is then used for counting CD4 cells. These CD4 cells are stained with acridine orange to make the cell nuclei visible for counting under a fluorescent microscope (Audu, 2002). In the Cytosphere system the monocytes are
not removed but appear different under the microscope, so the bead covered CD4 cells can be counted (Bergeron, 2002).

The quantitation of plasma viral ribonucleic acid (RNA) commonly referred to as viral load has provided valuable insights into the pathogenesis of HIV disease and activities of antiviral drugs (http://www.aidsnyc.org/natap). There are several approaches to measuring viral RNA which include branched deoxyribonucleic acid (bDNA) assay, which is based on a signal amplification method, and reverse transcriptase-polymerase chain reaction (RT-PCR) based on the reverse transcriptase methodology of amplifying the viral target. Two other methods, the nucleic acids sequence based amplification (NASBA) or quantitative competitive PCR (QC-PCR) share a common methodology of amplifying the viral target (http://www.aidsnyc.org/natap).

2.5 Commencement of Chemotherapy for HIV patients

There is variation in response to HIV infection and the different immune responses determine the progression of AIDS and also the rate of CD4 cell decline in the infected individuals. Disease progression can be considered in three stages: early immune depletion, during which the virus in blood is very low, HIV replication taking place mostly within the lymph nodes and CD4 count being more than 500 cells/mm$^3$; intermediate immune depletion, when the immune deficiency increases, infections start and persist or increase as the CD4 count drops, being between 500 to 200 cells/mm$^3$; and advanced immune depletion, when there is case definition of AIDS and having a CD4 count of less than 200 cells/mm$^3$ (Klatta, 1999). The variation between CD4 cells and viral load in HIV infected individuals in different regions will
dictate guidelines of when to initiate therapy. The decision to start therapy should be 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 count and viral burden (NASCOP, 2001).

According to published international guidelines, the following broad criteria guide the selection of patients for initiation of therapy: all patients with symptomatic HIV infection regardless of CD4 count and viral load levels; all patients with CD4 counts below 350 cells/mm$^3$ and finally all patients with a high viral load (i.e. above 30,000 copies/ml: Paula et al., 2001). The guidelines recommend that treatment be considered for patients in the intermediate range, that is plasma viral load between 10,000 and 30,000 copies/ml and CD4 cell counts between 350 and 500 cells/mm$^3$. Treatment of asymptomatic patients, with CD4 cell counts above 500 cells/mm$^3$ is generally deferred as long as the probability of significant immune system damage and of clinical progression of HIV infections remain low (Paula et al., 2001). An international AIDS society USA panel recommends starting management if CD4 cell counts is 200 cells/mm$^3$ in asymptomatic people with a CD4 count falling faster than 100 cells/mm$^3$ yearly or with a viral load above 50,000 to 100,000 copies/ml (Yeni et al., 2002). British HIV Association advises asymptomatic people with CD4 counts above 350 cells/mm$^3$ to defer treatment (Gretchen, 2003). Management should start when the count falls below 200 cells/mm$^3$ and may begin between 200 and 350 cells/mm$^3$, depending on the rate of the CD4 decline, symptoms, patient’s wishes and viral load (Gretchen, 2003; http://www.bhiva.org/guidelines.pdf). For poor and developing countries, the World Health Organization advise treating anyone with
AIDS, a CD4 count below 200 cells/mm$^3$, or a total lymphocyte count below 1,200 cells/mm$^3$ (http://www.who.docstore/hiv/scaling).

When randomized clinical trials provide strong evidence for treating patients with less than 200 CD4 cells/mm$^3$ (Gulick et al., 1997; Hammer et al., 1997; Cameron et al., 1998), the optimal time to initiate antiretroviral therapy among asymptomatic patients with CD4 counts more than 200 cells is not known. For individuals with more than 200 CD4 cells/mm$^3$, the strength of the recommendation for therapy must balance the readiness of the patient for treatment, consideration of the prognosis as determined by baseline CD4 count and viral load levels, and assessment of the risks and potential benefits associated with initiating antiretroviral therapy (Antony et al., 2002). Once the decision has been made to initiate antiretroviral therapy, the goals should be maximal and durable suppression of viral load, restoration and/or preservation of immunologic function, improvement of quality of life, and reduction of HIV-related morbidity and mortality (MOH, 2002). Results of therapy are evaluated primarily with plasma HIV RNA levels; these are expected to show a one – $\log_{10}$ decrease at eight weeks and no detectable virus (less than 50 copies/ml) at 4 - 6 months after initiation of treatment (Antony et al., 2002). CD4 counts should be measured at the time of diagnosis and generally every 3 - 6 months thereafter. These intervals between tests are merely recommendations and flexibility should be exercised according to the circumstances of the individual case. Plasma human immunodeficiency virus ribonucleic acid (HIV RNA) levels should also be measured immediately prior to and again 2-8 weeks after initiation of antiretroviral therapy (Mellors et al., 1997).
2.6 Mode of Action of Antiretroviral Drugs

The gold standard of antiretroviral therapy is highly active antiretroviral therapy (HAART), a combination of three or more antiretroviral drugs in the management of HIV infection (MOH, 2002). The ability of the patient to adhere to the regimen is essential for successful management. Excellent adherence has been shown to increase the likelihood of sustained virologic control, which is important for reducing HIV-related morbidity and mortality. Conversely, poor adherence has been shown to increase the likelihood of virologic failure and has been associated with increased morbidity and mortality (Carmona et al., 2002; Patterson et al., 2000). Poor adherence leads to the development of drug resistance, limiting the effectiveness of therapy (Walsh et al., 2000). There are 21 drugs approved for the management of HIV infections, including late infection (AIDS). They fall into 4 classes: Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIS), Non nucleoside reverse transcriptase inhibitors (NNRTIS), Protease inhibitors (PIS) and Fusion inhibitors.

Nucleoside or Nucleotide reverse transcriptase inhibitors NRTIS work by blocking the enzyme reverse transcriptase (Figure 2.2), which helps the virus make DNA from its RNA (http://www.cancer.org/docroot/CRI/content/CRI). Inside HIV particles are two strands of RNA and a unique enzyme, reverse transcriptase. After the virus particle enters the cell, reverse transcriptase copies (transcribes) RNA to DNA. First, the enzyme binds to an RNA strand; then it matches RNA nucleotides with a corresponding DNA nucleotide. The cell provides the nucleoside bases that are: adenosine, cytidine, guanosine, and thymidine. Cellular kinases convert them to active nucleotide by adding three phosphate radicals. Nucleoside reverse transcriptase inhibitors (NTRIS) mimic nucleosides (Gretchen, 2003). They too
become phosphorylated into the active form by cellular kinases. Nucleoside analogs lack the critical hydroxyl group at the point where nucleotides bind together. This altered structure prevents reverse transcriptase from adding more nucleotides to the DNA chain. If the enzyme adds an analog instead of a nucleotide, transcription stops, and the virus cannot replicate. The enzyme inserts the triphosphate form of the nucleoside analog instead of a cellular nucleotide into the growing DNA chain (Figure 2.2).

The structure of a nucleoside analog is modified so that another nucleotide cannot be linked to it, and elongation stops. Both nucleoside and nucleotide analog compete with cellular nucleotides for incorporation by reverse transcriptase into human immunodeficiency virus deoxyribonucleic acid (HIV DNA: Bart et al., 2000; Clumeck et al., 2001; Piscitelli and Gallicano, 2001 and Carr et al., 2002). Zidovudine\textsuperscript{R} (ZDV or AZT), an NRTI, was the first drug to show benefit in the management of AIDS. It is still one of the main drugs given. Other drugs in this class include abacavir (Ziagen\textsuperscript{R}), didanosine (Videx\textsuperscript{R}), emtricitabine (Emtriva\textsuperscript{R}), lamivudine (Epivir\textsuperscript{R}), stavudine (Zerit\textsuperscript{R}), tenofovir (Viread\textsuperscript{R}), and zalcitabine (Hivid\textsuperscript{R}; http://www.cancer.org/docroot/CRI/content/CRI). Adverse effects associated with NRTI use alone or in combination include lactic acidosis and severe hepatomegaly with steatosis, including fatal cases (Gretchen, 2003).
Figure 2.2: Mode of Action of Antiretroviral Drugs (Source, WHO)
These adverse effects are due to toxicity of the NRTIS on cellular mitochondria (Paula et al., 2001). Nucleoside reverse transcriptase inhibitors are eliminated primarily through the kidneys and do not interact in the liver with drugs metabolized by the hepatic cytochrome P450 system. Cytochrome P450 system is a set of liver enzymes that metabolize drugs and other substances. Nucleoside reverse transcriptase inhibitors can usually be given with Protease inhibitors (PIs) and Non- nucleoside reverse transcriptase inhibitors (NNRTIS) without adjusting the doses (Piscitelli and Gallicano, 2001). Two NRTIS generally form the backbone of most of these combinations (Paula et al., 2001).

Non-nucleoside reverse transcriptase inhibitors (NNRTIS) are the other group of antiretrovirals but they are chemically different from nucleoside reverse transcriptase inhibitors and act on a different part of the reverse transcriptase molecule (http://www.cancer.org/docroot/CRI/content/CRI). Instead of using the RNA polymerase enzyme to copy RNA to DNA, HIV relies upon a RNA dependent polymerase Reverse Transcriptase to copy RNA to DNA. Since this enzyme is specific to the virus, it must be brought into the cell upon infection. Two distinct subunits comprise the lopsided protein- p66 and p51 with the active site of transcription residing on the p66 subunit. Non-nucleoside reverse transcriptase inhibitors (NNRTIS) bind to a pocket near this active site. Once bound, NNRTIS cause the enzyme to change its shape, suppressing its transcription ability, severely inhibiting its function as a polymerase (Gretchen, 2003). Currently there are only three non-nucleoside reverse transcriptase inhibitors (NNRTIS) in the market namely: nevirapine (Viramune®), delavirdine (Rescriptor®) and efavirenz (Sustiva®) (http://www.cancer.org/docroot/CRI/content/CRI). All three approved NNRTIS
inhibit HIV-1 reverse transcriptase, but not the corresponding HIV-2 enzyme (Gretchen, 2003). The NNRTIS are mobilized by the cytochrome P450 system in the liver, primarily by the 3A4 isoform (CYP3A4; a liver enzyme that is part of the cytochrome system and metabolizes many drugs), and each NNRTI can inhibit or induce cytochrome P450 activity depending on the specific drug. Inhibiting the hepatic P450 metabolic pathway slows the breakdown of a drug, leading to increased drug concentration (Piscitelli and Gallicano, 2001). All NNRTIS may cause a skin rash; these rashes are generally mild and self-limited, though severe forms similar to a Stevens–Johnson syndrome have been reported. Non-nucleoside reverse transcriptase inhibitors may also cause elevation of serum aminotransferases and rare cases of fatal hepatitis have been reported (Paula et al., 2001).

Protease inhibitors (PIS) work by blocking the action of protease, so that new viruses are incomplete and cannot reproduce (http://www.cancer.org/docroot/CRI/content/CRI; Figure 2.2). The viral enzyme protease plays an essential role during the final stages of the viral life cycle. The enzyme, a protein product of HIV replication, is packaged by the new virus particles. As these new particles bud from the host cell, protease cleaves immature polyproteins into smaller, functional proteins that are necessary components of the mature virion. Without these proteins, the budding particles are not infectious. Protease inhibitors (PIs) bind to the enzyme’s active site and block its activity. Since these drugs act at a later phase of the viral life cycle, they have no protective effect on cells whose genomes already contain integrated provirus (Gretchen, 2003). Protease inhibitors include amprenavir (Agenerase®), atazanavir (Reyataz®, indinavir (Crixivan®), nelfinavir (Viracept®), ritonavir (Norvir®, saquinavir (Fortovase®), lopinavir® (combined with ritonavir and
called Kaletra), fosamprenavir (Levixa™) and tipranavir (Aptivus™). Some protease inhibitors are used along with a small dose of ritonavir, not because of its anti-HIV action, but because it slows the rate of drug metabolism. This is referred to as boosting a protease inhibitor, because it helps keep the drug levels in the body high (http://www.cancer.org/docroot/CRI/content/CRI). All approved PIs are metabolized by cytochrome P450 enzymes and may potentially interact with other drugs metabolized by the same pathway, and many of these interactions are clinically important. Protease inhibitors reduce the activity of cytochrome P450 system in the liver, specifically the 3A4 isoenzyme (CYP3A4; Piscitelli and Gallicano, 2001). Protease inhibitors cause significant side effects. They all cause gastrointestinal and metabolic disturbances, particularly lipodystrophy syndrome (lipoatrophy, central fat accumulation, and increased blood fats). Moreover, each drug has its own dose-limiting toxicity (Gretchen, 2003).

Entry/Fusion inhibitors work by blocking the entry of the virus into human cells. Only one drug in this class is available called Enfurvirtide (Fuzone™) and is given by injection (http://www.cancer.org/docroot/CRI/content/CRI). A HIV particle is studded with glycoproteins comprising pg120 and gp41 and each plays a unique role in infection. First, gp120 binds to the cell’s CD4 receptors. This initial binding flexes the complex and allow gp120 to attach to a second cell receptor, a chemokine receptor. Depending on the virus’s preference, the second receptor is either CCR5 or CXCR4. This second attachment induces another change in the complex, and frees up gp41 to unfold and attach onto the cell membrane. Refolding of gp41 brings the virus in contact with the cell membrane, resulting in virus-cell fusion and the
penetration of the HIV virion into the cell. Enfuvirtide interferes with entry by binding to gp41 and preventing the virus from fusing to the cell (Gretchen, 2003).

Combination drugs are fixed – dose combinations of the above drugs that help to reduce the number of pills a person has to take. For example, zidovudine and lamivudine are combined to form a pill called Combivir®; zidovudine and lamivudine and abacavir are put together for Trizivir®, abacavir and lamivudine are combined to form Epzicom® and tenofovir and emtricitibine are combined to make Truvada® (http://www.cancer.org/docroot/CRI/content/CRI).

The choice of a particular regimen remains individualized with consideration given to the strength of supportive data, the tolerability of the regimen, the potential for adverse effects, likely drug interactions, convenience and likelihood of adherence and the potential for alternative treatment options should an initial combination fail (Paula et al., 2001). Of the 21 antiretroviral drugs in use, some are recommended for use only when the first choice of drugs has failed (http://www.cancer.org/docroot/CRI/content/CRI). Antiretroviral drugs may need to be changed for either treatment failure or toxicity. Treatment failure can be evaluated clinically, immunologically using measurement of the CD4 count, and/or virologically by measuring viral loads (MOH, 2002). Toxicity is related to the inability to tolerate the side effects of the medication and to significant organ dysfunction that may result. This can be monitored clinically based on patient reports and physical examination, and may include a limited number of laboratory tests depending on the specific combination regimen that is utilized (Hammer et al., 2002).
2.7 Initiation of Antiretroviral Therapy in the Asymptomatic HIV Infected patients

Antiretroviral therapy should be started after both the patient and doctor explores the risks and benefits of the drugs being considered. The best time to start is not clear-cut, because HIV is an infection that usually progress slowly. Most doctors agree that antiretroviral therapy should begin when HIV infection is causing significant symptoms, regardless of CD4 count and viral load, and when the CD4 level is at or below 200 cells/mm$^3$ (some doctors and guidelines recommend starting treatment if the CD4 count is below 350 cells/mm$^3$), even if there are no symptoms some doctors will also treat people with viral load over 100,000 copies/ml of blood, regardless of CD4 count or symptoms (http://www.cancer.org/docroot/CRI/content/CRI).

2.7.1 Risks and Benefits of Early Initiation of Therapy

The potential benefits of early therapy include earlier suppression of viral replication, preservation of immune function and decrease in the risk of viral transmission (Antony et al., 2002). Risks of early therapy include greater cumulative drug-related adverse effects, earlier development of drug resistance, if viral suppression is suboptimal, and limitation of future antiretroviral treatment options (NASCOP, 2001).

2.7.2 Risks and Benefits of Delayed Therapy

The potential benefits of delayed therapy include minimization of treatment-related negative effects on quality of life and drug-related toxicities, preservation of treatment options and delay in the development of drug resistance. Potential risks of delayed therapy include the possibility that some damage to the immune system that might otherwise be salvaged by earlier therapy is irreversible, the possibility that suppression of viral replication may be more difficult at a later stage of disease and
the increase risk of HIV transmission to others during a longer untreated period (Antony et al., 2002).

The decision to begin therapy is complex and must be made in the setting of careful patient counseling and education. The factors that must be considered in this decision are: the willingness, ability and readiness of the individual to begin therapy; the degree of existing immunodeficiency as determined by the CD4 count; the risk of disease progression as determined by the CD4 count and viral load; the potential benefits and risks of initiating therapy and the likelihood of adherence to the prescribed treatment regimen (Paterson et al., 2000; Carmona et al., 2002). Starting lifelong antiretroviral therapy should not be seen as an emergency measure. Starting ARVs before the patient really understands the overriding importance of strict, long term adherence treatment, will actually result in a worse outcome for that individual, than delaying to give time for good adherence counseling and education (MOH, 2004).

2.8 Rationale for Stopping Antiretroviral Therapy

There are multiple reasons for temporary or permanent discontinuation of antiretroviral therapy. If there is a need to discontinue any antiretroviral medication, clinicians and patients should be aware of the theoretical advantages of stopping all antiretroviral agents simultaneously, rather than continuing one or two agents, to minimize the emergence of resistant viral strains (Antony et al., 2002). Various reasons for stopping treatment in HIV infected patients for short periods of time have been hypothesized (MOH, 2002). One proposal was that structured treatment interruptions (STIs) would provoke the sturdiest immune response in patients treated
shortly after primary infection, because quick treatment would preserve CD4 cells that promote the specific immune defence against HIV infection (Walker, 2002). Studies in people who had been successfully treated during chronic infection hoped that temporary, controlled ‘autoinoculation’ with one’s own virus during treatment breaks would jog the immune system’s faulty memory into recognizing HIV (Walker, 2002). A vigorous immune response against HIV might put the virus into remission and allow patients eventually to stop antiretroviral completely (Gretchen, 2003).

Before initiating STIs, a patient should have immune reconstitution within acceptance limits, i.e. viral load below 50 copies/ml for at least a period of one year. On commencing STI, the viral load and CD4 and CD8 values should be monitored on every three months’ intervals (MOH, 2002). Studies of structured and unstructured breaks by people with chronic infection show that CD4 counts usually fall quickly during prolonged interruptions, while viral loads climb to, or above, pretreatment baselines (Tebas et al., 2002; Mussin et al., 2002; Tesiorowski et al., 2001; Youle et al., 2000). Evidence supports interrupting treatment do not improve immunity, but simply reduce toxicity and improve quality of life. A break from antiretroviral side effects may be the most reasonable gain to anticipate from STIs during chronic infection (Gretchen, 2003). Initiation of STI to control or prevent some long term toxicities associated with HAART therapy might be useful (MOH, 2002). One study did yield evidence of decreased toxicity in people taking antiretrovirals every other week (Dybul et al., 2001). Median triglycerides, total cholesterol and low-density lipoprotein cholesterol dropped significantly.
There is not enough data to support the fact that the temporary cessation of therapy has any significant impact on side effects such as elevated liver function, or peripheral neuropathy or nutritional disorders (MOH, 2002). For some people, switching on and off therapy every week or every few months could worsen adherence because of the complex schedule and difference in quality of life during the on and off periods (Gretchen, 2003). Poor adherence had been identified as a leading cause of drug failure in patients receiving antiretroviral therapy. Stopping therapy in these patients, until at least these adherence issues are dealt with may prove to be a short-term preventive solution to a long-term dilemma of multiple drug resistance (MOH, 2002).

Salvage structured treatment interruption is directed at patients whose virus has developed significant antiretroviral drug resistance and who have persistent viremia and relatively low CD4 counts despite receiving therapy. The theoretical goal of STI in this patient population is to allow for the re-emergence of HIV that is susceptible to antiretroviral therapy (Antony et al., 2002). Although HIV that was sensitive to antiretroviral agents was detected in the plasma of many individuals following several weeks to months of interrupting treatment, the emergence of drug-sensitive HIV was associated with a significant decline in CD4 cells and a significant increase in plasma viremia, suggesting improved replicative fitness and pathogenicity of wild type virus (Deeks et al., 2001). In addition, drug resistant HIV persisted in CD4 cells.

Structured treatment interruptions (STIs) for the reduction of total time of drugs are directed at individuals who have maintained suppression of plasma viremia below the limit of detection for prolonged periods of time and who have relatively high CD4 counts. The theoretical goal of STI is to allow several short burst of viral replication
to augment HIV-specific immune responses (Rosenberg et al., 2000; Fagard et al., 2001; Dybul et al., 2001). Structured treatment interruptions for the purpose of less time on therapy utilizes predetermined periods of long or short cycle intermittent antiretroviral therapy (Fagard et al., 2001). Potential risks include a decline in CD4 counts, an increase in transmission and the development of drug resistance (Antony et al., 2002).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site and Study Population

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 kilometres 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 Counselling 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.
3.2 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 (Appendix 1).

3.3 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 (Appendix 2). 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, \( n = \frac{Z^2pqD}{d^2} \) 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 formular was used (Fisher et al., 1998).

\[
nf = \frac{n}{1 + n/N} = \text{sample size estimate} = 5
\]

\[
n = \text{calculated sample size}
\]

\[
N = \text{monthly average attendance at the VCT.}
\]

The sample size was estimated as follows:
\[ nf = 1 + \left(\frac{400}{100}\right) = 80 \]

A sample size of 80 patients was used.

3.4 Screening for Human Immunodeficiency Virus

3.4.1 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. 3.1).
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. 3.2).
3.4.2 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.

3.5 Data on Opportunistic Infection

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

3.6 CD4 Count Determination

CD4 counts were carried out using Beckton Dickson (BD) FACSCCount system (BD Biosciences, USA) according to the manufacturers’ protocol (David et al., 2004). Beckton Dickson FACSCCount is a complete system incorporating instrument, reagents, controls and software. It utilizes a direct two-colour immunofluorescence 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 FACSCCount 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.

3.7 Viral Load Determination

Viral load was determined using ExaVir Load kit (Cavidi Tech AB, Sweden) according to the protocol provided by the manufacturer (Malmstem et 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 into 1.2ml 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.

3.8 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.

3.9 Data Management.

CD4 counts, viral loads as indications of patients’ responses were analysed using Chi-square 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.
CHAPTER FOUR: RESULTS

4.1 Screening for HIV 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 4.1). None of the female patients was pregnant.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 21</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>21 – 25</td>
<td>2</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>26 – 30</td>
<td>3</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>31 – 35</td>
<td>2</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>36 – 40</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>More than 40</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>68</td>
<td>80</td>
</tr>
</tbody>
</table>

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.

4.2 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 4.1). 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 4.1). 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 4.1). 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 4.1).
Table 4.2: CD4 Levels and corresponding clinical manifestations of patients

<table>
<thead>
<tr>
<th>CD4 levels</th>
<th>Mean CD4 count</th>
<th>Number of patients</th>
<th>Opportunistic infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 100</td>
<td>54</td>
<td>27 (33.75%)</td>
<td>Chronic weakness, Chronic diarrhoea, Kaposi's sarcoma, Candidiasis of the oesophagus, Tuberculosis, Pneumonia</td>
</tr>
<tr>
<td>cells/mm³ of blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 – 200 cells/mm³</td>
<td>151</td>
<td>42 (52.5%)</td>
<td>Persistent/consistent fever, Pneumonia, Tuberculosis, Chronic diarrhoea, Oral candidiasis</td>
</tr>
<tr>
<td>of blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;200 cells/mm³</td>
<td>210</td>
<td>11 (13.75%)</td>
<td>Persistent generalized lymphadenopathy, Oral candidiasis, Recurrent upper respiratory infections, Herpes Zoster</td>
</tr>
<tr>
<td>of blood</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 4.1).
4.3 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 4.2). 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 lymphadenopathy (Table 4.2). 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 diarrhoea and oral candidiasis (Table 4.2). 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 diarrhoea, Kaposi’s sarcoma, candidiasis of the oesophagus, tuberculosis, Herpes simplex and pneumonia (Table 4.2).
Table 4.3: Viral load and corresponding clinical manifestations of patients

<table>
<thead>
<tr>
<th>Viral load levels</th>
<th>Mean viral load</th>
<th>Number of patients</th>
<th>Opportunistic infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 50,000 copies/ml of plasma</td>
<td>46,941</td>
<td>26 (32.5%)</td>
<td>Persistent generalized lymphadenopathy Recurrent upper respiratory infections Oral candidiasis Herpes zoster</td>
</tr>
<tr>
<td>50,000-100,000 copies/ml of plasma</td>
<td>63,606</td>
<td>7 (8.75%)</td>
<td>Persistent/consistent fever Pneumonia Tuberculosis Chronic diarrhoea Oral candidiasis</td>
</tr>
<tr>
<td>&gt;100,000 copies/ml of plasma</td>
<td>308,796</td>
<td>47 (58.75%)</td>
<td>Chronic weakness Chronic diarrhoea Kaposi's sarcoma Candidiasis of the oesophagus Tuberculosis Herpes simplex Pneumonia</td>
</tr>
</tbody>
</table>

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 4.2).
Figure 4.2: Mean viral load (RNA copies) during chemotherapy.
4.4 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 4.3). 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 4.3). Among the patients with CD4 counts between 100-200 cells/mm³ 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 4.3). For the patients with more than CD4 counts 200 cells/mm³ 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 4.3).
Table 4.4: Effect of chemotherapy on CD4 count two weeks post chemotherapy

<table>
<thead>
<tr>
<th>Effect of Chemotherapy on CD4 counts</th>
<th>Baseline CD4 Counts</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 cells</td>
<td>100 – 200 cells</td>
</tr>
<tr>
<td>Increased</td>
<td>21 (77.8%)</td>
<td>35 (83.3%)</td>
</tr>
<tr>
<td>Decreased</td>
<td>5 (18.5%)</td>
<td>6 (14.3%)</td>
</tr>
<tr>
<td>No change</td>
<td>1 (3.7%)</td>
<td>1 (2.4%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>27 (33.7%)</td>
<td>42 (52.5%)</td>
</tr>
</tbody>
</table>

After six weeks of chemotherapy, seventy four patients (92.5%) had increased CD4 counts while six patients (7.5%) had decreased CD4 counts (Table 4.4). 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 4.4). 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 4.4). 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 4.4).
### Table 4.5: Effect of chemotherapy on CD4 count six weeks post chemotherapy

<table>
<thead>
<tr>
<th>Effect of Chemotherapy on CD4 counts</th>
<th>Baseline CD4 Counts</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 cells</td>
<td>&gt; 200 cells</td>
</tr>
<tr>
<td>Increased</td>
<td>25 (92.6%)</td>
<td>8 (72.7%)</td>
</tr>
<tr>
<td>Decreased</td>
<td>2 (7.4%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>27 (33.7%)</td>
<td>11 (13.8%)</td>
</tr>
</tbody>
</table>

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 4.5). 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 4.5). 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 4.5).

### Table 4.6: Effect of chemotherapy on CD4 count ten weeks post chemotherapy

<table>
<thead>
<tr>
<th>Effect of Chemotherapy on CD4 counts</th>
<th>Baseline CD4 Counts</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 cells</td>
<td>&gt; 200 cells</td>
</tr>
<tr>
<td>Increased</td>
<td>26 (96.3%)</td>
<td>10 (90.9%)</td>
</tr>
<tr>
<td>Decreased</td>
<td>- (%)</td>
<td>1 (9.1%)</td>
</tr>
<tr>
<td>No change</td>
<td>1 (3.7%)</td>
<td>- (%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>27 (33.7%)</td>
<td>11 (13.8%)</td>
</tr>
</tbody>
</table>

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 4.6). Among the patients with CD4 counts less than 100 cells/mm³ 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 4.6). Among the patients with CD4 counts between 100-200 cell/mm³ 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 4.6). For the patients with CD4 counts more than 200 cells/mm³ 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 4.6).

Table 4.7: Effect of chemotherapy on CD4 count fourteen weeks post chemotherapy

<table>
<thead>
<tr>
<th>Effect of Chemotherapy on CD4 counts</th>
<th>Baseline CD4 Counts</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 cells</td>
<td>100 - 200 cells</td>
</tr>
<tr>
<td>Increased</td>
<td>26 (96.3%)</td>
<td>37 (88.1%)</td>
</tr>
<tr>
<td>Decreased</td>
<td>-</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td>No change</td>
<td>1 (3.7%)</td>
<td>2 (4.8%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>27 (33.7%)</td>
<td>42 (52.5%)</td>
</tr>
</tbody>
</table>

4.5 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/mm³ 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/mm³ 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 4.3).

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 4.3; $P<0.01; t = 19.7332$) than the patients with less than 100 cells/mm$^3$ and patients with more than 200 cells/mm$^3$ of blood.
Figure 4.3: CD4 profile during chemotherapy. Patients categorized according to the level of CD4 counts.

* Asterisk means significantly better response in this category of patients than the patients in the other two categories. $P<0.01; t=19.7332$. 
4.6 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 4.7). 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 4.7).

<table>
<thead>
<tr>
<th>Effect of Baseline Viral Load</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50,000 copies/ml</td>
<td>50,000 - 100,000 copies/ml</td>
</tr>
<tr>
<td>Decreased</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Increased</td>
<td>12 (46.1%)</td>
</tr>
<tr>
<td>No change</td>
<td>1 (3.9%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>26 (32.5%)</td>
</tr>
</tbody>
</table>

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 4.8). 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 4.8).

Table 4.9: Effect of chemotherapy on viral load six weeks post chemotherapy

<table>
<thead>
<tr>
<th>Effect of Chemotherapy on viral load</th>
<th>Baseline Viral Load</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50,000 copies/ml</td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>12 (46%)</td>
<td>34 (42.5%)</td>
</tr>
<tr>
<td>Increased</td>
<td>14 (54%)</td>
<td>46 (57.5%)</td>
</tr>
<tr>
<td>Percentage of decrease (%)</td>
<td>46.15</td>
<td>36.17</td>
</tr>
<tr>
<td>Total patients</td>
<td>26 (32.5%)</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>7 (8.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 (36%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 (64%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47 (58.7%)</td>
<td></td>
</tr>
</tbody>
</table>

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 4.9). 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 4.9). 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 4.9). 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 4.9).
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 4.10). 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 4.10). 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 4.10). 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 4.10).
4.7 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 (Figure 4.4).

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 4.4; \( P<0.001; t = 460.7554 \)) than the patients with 50,000-100,000 copies/ml and less than 50,000 copies/ml categories.
Figure 4.4: Viral load profile during chemotherapy. Patients categorized according to the level of viral load.

* Asterick means significantly better response in this category of patient than the other two categories. $P< 0.001; t = 460.7554$. 
4.8 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 4.5; \( P<0.001; r = -0.992 \)), 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/mm\(^3\) 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 4.5: Mean CD4 counts and mean viral loads for all the patients during chemotherapy.

* Asterick means strong inverse correlation between viral load and CD4 counts. \( P<0.001; r = -0.992 \)
4.9 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. (Figure 4.6).

Figure 4.6: Response to Chemotherapy in terms of CD3 and CD8 counts

* Asterick means strongly inversly correlated
4.10 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 4.12). 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/mm³ of blood at the baseline rose from 0.04 to 0.10 during the fourteen weeks of chemotherapy (Figure 4.12) while CD4:CD8 ratio for the patients with CD4 counts between 100-200 cells/mm³ of blood at the baseline rose from 0.10 to 0.19 during the fourteen weeks of chemotherapy (Figure 4.12). The CD4:CD8 ratio for the patients with CD4 counts more than 200 cells/mm³ of blood at the baseline increased from 0.18 to 0.40 during the fourteen weeks of chemotherapy (Figure 4.7).

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/mm³ of blood at the baseline were found to have a significantly higher change (Figure 4.7; P < 0.001; t = 39.91063) in CD4:CD8 ratio than patients with less than 100 cells/mm³ and patients with 100-200 cells/mm³ of blood at the baseline categories.
Figure 4.7: Effect of chemotherapy on CD4: CD8 ratio

* Asterick means significantly better response in this category of patients than the other two categories. P<0.001; t = 39.91063.
CHAPTER FIVE: DISCUSSION

5.1 Overview

Acquired immunodeficiency syndrome (AIDS) represents a global health crises 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 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).

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,
The individuals in the current study were given two nucleoside RTIs (Lamivudine+ Stavudine) and non-nucleoside RTI (Nevirapine).

CD4 counts and 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 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 and viral load 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.

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 and viral loads. They were advised on the need to commence antiretroviral drugs and were made to understand the need to have their CD4 counts and viral loads monitored to assess the effect of chemotherapy.
5.2 HIV Screening Tests

The data reported in this study was obtained from individuals who 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 involved, their purpose and how any findings were to be used. After pre-testing counseling all individuals consented for an HIV test which would confirm diagnosis that was important for this study.

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 parallel 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.

After testing positive for HIV infection, the patients were counseled. They were told of how its possible to lead a normal life despite being HIV positive, how they could improve their immune status by feeding on proper diet and getting medication to avoid opportunistic infections, how starting antiretroviral therapy (ART) and being committed to treatment could improve the quality of their lives and how to live a positive life.

5.3 Opportunistic Infections

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 and viral loads more than 100,000 copies/ml of plasma 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 and had viral loads between 50,000 -100,000 copies/ml of plasma commonly presented with chronic diarrhoea, tuberculosis, pneumonia and consistent fever while individuals whose CD4 counts were more than 200 cells/mm$^3$ of blood and viral loads less than 50,000 copies/ml of plasma commonly presented with persistent generalized lymphadenopathy, 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 et al, 2001; Tafteng et al., 2007).
5.4 Response to Chemotherapy

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 nelfinavir 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 22 cells/mm$^3$ at two weeks post treatment while the highest mean increase in CD4 counts was 61 cells/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 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 while others increased viral load 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) and increase in viral load during treatment has also been reported (Hoffman et al., 1999; Tafteny et al., 2007). In this study, the decrease in CD4 count and increase in
viral load 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.

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 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.
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.project.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 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.
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) Increases in CD4 counts and viral load reductions were observed two weeks after initiation of chemotherapy.

ii) Better responses to chemotherapy were observed in the patients who started the treatment with medium CD4 counts (100 – 200 cells/mm$^3$) and higher viral loads (more than 100,000 copies/ml) during the period of chemotherapy.

iii) CD3 counts increased with chemotherapy while CD8 counts decreased with chemotherapy.

iv) CD4:CD8 ratio increased with chemotherapy.

v) CD4 counts and viral loads were found to be strongly inversely correlated. As the CD4 counts increased, viral loads decreased.

vi) CD4 counts or viral loads are predictive of the benefits of chemotherapy and depending on the facility available either of the parameters can be used to monitor responses to chemotherapy.

6.2 Recommendations

i) Antiretroviral therapy should commence before CD4 counts are too low (less than 100 cells/mm$^3$ of blood).

ii) Measure of CD4 counts or viral loads are predictive of response to ARVs.

iii) Opportunistic infections are suggestive of CD4 counts and viral loads (useful data for clinicians).
iv) This kind of study should be carried out for a longer period of time not just fourteen weeks to monitor the effects of antiretroviral drugs on the parameters studied, and in particular to enable achieve undetectable viral loads.

v) This kind of study should be repeated in other different settings of other parts of the country to qualify the correlation and predictive value of either of the parameters reported here.
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Provincial General Hospital records (2005).


APPENDICES

APPENDIX I

APPROVAL TO CONDUCT THE STUDY AT NAKURU GENERAL HOSPITAL

MINISTRY OF HEALTH

Telegrams: "PROVMED", Nakuru
Telephone: Nakuru 215580-90
When replying please quote
Ref. No. ........................................
and date

RII/VOL.1/05
29th September, 2005

Kenyatta University
Department of Biological Sciences
P O Box 43844
NAIROBI

Dear Sir/Madam,

RE: CONSENT TO CARRY OUT RESEARCH IN PGH
MISS JANE MUGWE

This is to inform you that the above named has been given consent to carry out her
immunological research in this Institution.

Thank you.

Yours faithfully,

DR. B. R. OMBITO
MEDICAL SUPERINTENDENT
PGH NAKURU
APPENDIX II

PATIENT'S CONFIDENTIALITY QUESTIONNAIRE INSTRUCTIONS:

1. DO NOT WRITE YOUR NAME ON THIS QUESTIONNAIRE.

2. ALL INFORMATION YOU GIVE WILL BE TREATED AS CONFIDENTIAL.

3. WHERE THERE ARE BRACKETS, ( ), PUT A TICK, ( ), WHERE APPROPRIATE.

4. GIVE SHORT ANSWERS WHERE NECESSARY.

N.B. Your contribution will be highly appreciated.

1. Name of your village / estate ____________________________________________________________

2. Division ___________________________ District _____________________________

3. Gender: Male ( ), Female ( ).

4. How old are you? ___________________________

5. Marital Status: Single ( ), Married ( ), Widow/Widower ( ), Separated ( ).

6. What is your occupation? ___________________________

7. Are you willing to undergo HIV test? Yes ( ), No ( ).

8. Are you psychologically prepared for whichever outcome of the result? Yes ( ), No ( ).

9. It's advisable to start ART if results are HIV - Positive after certain clinical diagnostic tests. Would you accept this if the results suggests so? Yes ( ), No ( ).

FOR OFFICIAL USE ONLY

PATIENT: ________________________________

INTERVIEWED BY: ________________________________