EVALUATION OF EARLY IMMUNOLOGICAL RESPONSE AMONG PATIENTS ON FIRST LINE HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY (HAART) AT KENYATTA NATIONAL HOSPITAL

A DISSERTATION SUBMITTED AS PART OF FULLFILMENT OF REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI.

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DECLARATION

I certify that this dissertation is my own original work and that there has been no presentation of this work at any other university for the award of a degree.

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DEDICATION

This thesis is dedicated to my sister EVELYNE and all HIV/AIDS patients who are positively living with the disease not forgetting those who died from AIDS prior to availability of HAART in this country.
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ABSTRACT

Objective: Introduction of highly active antiretroviral therapy in 1996 has revolutionized the care of patients infected with HIV. Complete viral suppression in patients on HAART results in immunological response evidenced by an increase in CD4+ T-cell count. Early good immunological response to antiretroviral therapy portends good clinical outcome in AIDS patients. The use of first line HAART has been scaled up in this country but there is paucity of data on immunological response to such therapies. This study evaluated early immunological response among patients on first line HAART attending to CCC at KNH between the year 2003 and June 2007.

METHODOLOGY

This was a retrospective analytical study conducted among patients on first line HAART attending the CCC at Kenyatta National Hospital. A list of files of all patients on first line HAART was obtained from CCC pharmacy and Demographic data, baseline CD4+ T-cell count and CD4+ T-cell count at six months of treatment extracted from files that met our inclusion criteria. CD4+ cell change was used to assess Immunological response with good response being defined as a change in CD4+ cell count of 50 cells/mm³ or more from the baseline level at six months. Data was analyzed using SPSS version 15.0.
RESULTS
One thousand six hundred and seventy-eight patients were on first line therapy within the study duration. Out of these, 780 patients did not meet the inclusion criteria while 98 files could not be retrieved from the records department leaving 800 patients for analysis. The study had more females than males with male to female ratio of 1:1.4. The mean age for the group was 38.1 years with median of 37 years. Fifty seven percent of patients were in WHO stage three. The mean baseline CD4+ cell count was 127.2 cells/mm³ while mean CD4+ cell count at six months of therapy was 239.81 cells/mm³. Good immunological response was recorded in 77.6% of the study population and this was not influenced by demographic factors namely age and sex. CD4+ T-cell count at six months was significantly associated with baseline CD4+ cell count with an R^2 square of 0.62 at 95% mean prediction interval on linear regression.

CONCLUSION
This study demonstrated good immunological response among patients on first line HAART in Kenyatta National Hospital. Therefore, first line HAART regimen currently in use (stavudine, lamivudine and nevirapine or efavirenz) is effective if immunological response alone is considered. This study has also demonstrated the utility of Baseline CD4+ count in predicting CD4+ T cell count at six months of therapy.
1.1 EPIDEMIOLOGY

Infection with human immunodeficiency virus has grown to pandemic proportions resulting in 65 million infections and 25 million deaths since the first case was diagnosed in 1981, and the virus isolated in 1983(1-3).

According to UNAIDS/WHO 2006 AIDS epidemic update report, an estimated 39.5 million people were living with HIV with 4.3 million new infections being recorded during that year (4). However, new data released for the year 2007 shows that the global HIV prevalence—the percentage of people living with HIV—has leveled off and that the number of new infections has fallen partly due to the impact of HIV/AIDS programmes world wide (5).

During the year 2007, the total number of persons living with HIV/AIDS was estimated to be 33.2 millions with 2.5 million new infections and 2.1 million deaths reported world wide (5).

Though this is commendable, the prevalence of HIV/AIDS in some parts of the world is still rising. Since 2001 when the United Nations signed a declaration of commitment on HIV/AIDS, the number of people living with HIV in Eastern Europe and Central Asia has increased by more than 150% (from 630,000 to 1.6 million in 2007). In Vietnam, the number has more than doubled while Indonesia currently has the fastest growing epidemic (5). Global HIV incidence is now thought to have peaked in the late 1990’s at over 3 million as opposed to current rates of 2.5 million, which translates to an average of more than 6800 new cases each day. This change reflects natural trends in the epidemics as well as efforts on prevention that are now bearing fruits (5).

The number of people dying from AIDS-related illness has declined in the last two years due in part to the life prolonging effects of antiretroviral therapy. However, AIDS is still among the leading causes of death globally and remains the primary cause of death in Africa (5).

In the year 2007, Sub-Saharan Africa registered a drastic reduction in new HIV infection recording 1.7 million cases compared to 2.8 million cases recorded in
2006 (4-5). However, the region continues to be the most severely affected, shouldepering 68% of the total global number of people living with HIV-AIDS, which translates to 22.5 million people (5).

Transmission in this region is mainly through heterosexual contact, and women are more affected than men are. Southern Africa is the epicenter of the epidemic with all countries in the region except Angola having an estimated adult (15-49 years) HIV prevalence exceeding 10%. In Botswana, Lesotho, Swaziland and Zimbabwe, the estimated adult prevalence exceeds 20%. South Africa with a prevalence of 18.8% and 5.5 million people living with HIV, has, along with India, the largest number of persons living with HIV in the world (6).

In contrast, West Africa is relatively less affected even though some countries such as Cote d’vore and Nigeria have a rising prevalence (7). Infection rates in East Africa once the highest on the continent, hover above those of the west but have been exceeded by rates in the South. In Uganda, the estimated prevalence rate in 2005 fell to 5% from an all high of 15% in the 1990’s (7).

Two decades down the line since the first case was described in Kenya by Obel A.O and Okello (8-9), HIV/AIDS still remains a big burden for the country in its bid for social and economic development (10). The number of people living with HIV/AIDS in Kenya is estimated to be one million with most of them being in the age bracket of 15-49 years. In this country, HIV epidemic peaked in the year 1997/1998 with an overall HIV prevalence of around 10%. However, prevalence rate in adults have been declining, standing at 5.1% by December 2006 (12).

Women in Kenya are particularly vulnerable to HIV and AIDS. Of the 1.1 million adults infected with HIV, twice as many women as men are positive with the prevalence in women aged 15-49 years being 6.7% compared to 3.5% in men of the same age group. (11-12). Urban areas are more affected by the scourge than rural areas with prevalence rates of 8.3% and 4% respectively (12). Adult death rates peaked at 120,000 in 2003, and this would have remained at this level if it had not been for the expanding delivery of Anti Retroviral drugs. In 2006, the annual AIDS mortality rate had reduced to 85,000 implying that ART programme had averted about 57,000 deaths since 2001 (12).
The causative agent in HIV/AIDS is Human Immunodeficiency Virus that belongs to the family of human retroviruses (Retroviridae) and the sub-family of Lentivirus whose hallmark is the reverse transcription of its genomic RNA to DNA by the enzyme Reverse transcriptase (13). There are two types of HIV virus namely HIV-1 and HIV-2. HIV-1 has a worldwide distribution while HIV-2 is mainly endemic in West Africa.

Full genetic sequencing and molecular characterization has revealed three distinct genetic groups (classes) designated M, N and O. Group M is further subdivided into nine clades designated A, B, C, D, F, G, H, J and K. Clade B is mainly found in the USA and Europe while C is predominantly in South Africa, India and China. In Kenya the pre-dominant clades are A and D (2).

Transmission of HIV is pre-dominantly through heterosexual contact even though intravenous drug use contributes significantly to the spread. The virus can also be transmitted through blood and blood products, needle pricks and vertically from mother to child (9).

1.2 PATHOGENESIS OF HIV/AIDS

HIV virus has an icosahedra structure containing numerous external spikes formed by two major envelope proteins, the external gp120 and the trans-membrane gp41. The envelope encloses the inner membrane and the p24 core protein (capsid) within which is contained the genomic RNA and enzymes. HIV virus primarily targets cells that exhibit CD4+ antigen on their surface. These include CD4+ T-cells, macrophages, dendritic cells as well as follicular cells in the lymph nodes. HIV-1 entry process is initiated by binding of HIV glycoprotein gp120 to the CD4+ molecule, which acts as a receptor, on the surface of the target cell via a portion of its Vi region near the N terminal. This results in the transformation of gp120 that exposes a previously concealed co-receptor binding site. The co-receptor can be either CCR5 or CXCR4. Subsequent interaction of gp120 with either of these co-receptors results in a conformational change in another HIV glycoprotein gp41, which leads to the insertion of the N-terminal end
of gp41 into the cellular membrane. This results in the fusion of the virus with the cellular membrane of the host cell, allowing viral entry into the target cell (14). Reverse transcriptase, which is, contained in the infecting virion then catalyses the reverse transcription of the genomic RNA into double stranded DNA. This translocates into the nucleus where it is integrated into the host chromosomes through the action of virally encoded integrase. Activation of the pro-virus results into transcription of the DNA to either genomic RNA or mRNA. The mRNA is translated into proteins that undergo modification through glycosylation, myristylation, phosphorylation and cleavage. New viral particle is formed by the assembly of HIV proteins, enzymes and genomic RNA at the plasma membrane of the cell. This is followed by budding off of the virion from the cell surface in the process acquiring its external envelope (14-15). Disruption of the cell membrane results in osmotic disequilibria hence cell death.

The hallmark of HIV disease is the immunodeficiency that results from depletion and dysfunction of CD4+ T lymphocytes (14). The rate of CD4+ cell depletion is directly linked to the rate of viral replication (viral load) (16). Both cytopathic and apoptotic mechanisms have been implicated in the destruction of CD4+ cells. Single cell killing is the predominant HIV-1 induced cytopathic effect. This results from accumulation of un-integrated viral DNA or from inhibition of cellular protein synthesis after HIV infection (17-18). Syncytia formation involves fusion of the cell membrane of an infected cell with resultant formation of giant multinucleated cells. The other mechanism of functional and quantitative depletion of CD4+ Lymphocytes is the induction of anergy signals. Complexes of gp120 and antibody bind to CD4+ molecule causing CD4 cells to become refractory to further stimulation through the activation of their CD3 molecules (17). A negative signal is delivered to CD4+ T-cell after their component CD4+ molecule reacts with gp120 or gp120-anti-gp120 complexes. In this regard anti gp120, antibodies have been detected on CD4+ T lymphocytes in patients with AIDS (17).

Programmed cell death or apoptosis is a normal mechanism of cell death that was originally described in the context of the response of immature thymocytes
to cellular activation (17-18). It is a mechanism whereby the body eliminates auto-reactive clones of T-cells. It has recently been suggested that both quantitative and qualitative defects in CD4+ cells in patients with HIV infection may be the result of activation induced cell death and apoptosis (17). HIV surface glycoprotein (gp120) and transactivation protein Tat, singly or in combination can activate apoptosis in CD4+ T cells. The T-cell lymphocyte subset is helpful in monitoring the course of HIV infection as it produces quantitative abnormality in all populations of the immune system. The helper lymphocytes designated as CD4+ cells decrease over time for they are primary targets of HIV. Lymphocytes with a suppressor function designated as CD8 are not decreased and may initially be increased. Abnormalities in numbers of CD4 and CD8+ T - lymphocyte subsets and the CD4+/CD8+ ratio were used early in the AIDS epidemic to help define persons affected with AIDS before a screening test for HIV-1 was made available. A low number of CD4+ Lymphocytes alone or in combination with decreased CD4/CD8 ratio and absolute lymphocyte count can be useful as a predictor of disease progression. In persons six years of age or older with HIV infection, a CD4/CD8 ratio of less than 1, a CD4+ count of less than 500/mm ³ and a total lymphocyte count of less than 1500/mm ³ indicate a poor prognosis (19-20).

Evaluation of the immune system in HIV is complicated by the progressive nature of the infection with a constantly changing immunological status. A variety of acute and chronic infections may be associated with the depletion of CD4+ T cells. The decrease in CD4 lymphocytes and the increase in CD8 lymphocytes observed in AIDS are also reported in other viral infections, only that the T lymphocyte alterations in response to HIV are progressive and irreversible (21). Depressed cell mediated immune function can also occur as a chronic complication of Histoplasmosis which is manifested by low CD4+ T - cell count and a low of CD4+/CD8+ ratio. CD4+ lymphocytopenia has been associated with the use of certain medications including corticosteroids and chemotherapeutic agents (22). Diurnal variation in CD4+ T - cell count of more than 100cells/mm³
that does occur hence low levels may be recorded in the same patient depending on the time of sample collection (22-24).

1.3 HIV INFECTION AS A DISEASE

Infection with HIV is characterized by a high rate of viral replication within CD4+ cells and progressive loss of these cells. CD4+ cells in HIV infected patients fail to respond to HIV protein shortly after infection and loose their antigen specific responsiveness to opportunistic pathogens and to recall antigens. This is shown by a loss of antigen specific T cells proliferation. Reduced functions of these cells and the subsequent breakdown of cellular immunity contribute to the emergence of opportunistic infections and may facilitate HIV replication. Although the course of HIV infection may vary somehow, among individual patients a common pattern of development has been recognized (18). Primary infection is followed by the development of detectable humoral and cellular immune responses to the virus and a prolonged period (median of ten years) of clinical latency, during which time the patient is usually asymptomatic, followed by the appearance of constitutional signs and symptoms (18). During the early period after primary infection, there is widespread dissemination of the virus and sharp decline in the number of CD4+ T cells in the blood. Immune response to HIV ensues with decrease in detectable viraemia followed by prolonged period of latency. With progressive deterioration of the immune system that occurs in most patient's clinically apparent disease or an acquired immunodeficiency syndrome (AIDS) defining illnesses eventually develop. This marks the end of a stage of immunopathogenetic events that began at the time of primary infection.

Various organizations have come up with diagnostic criteria and staging in patients with HIV. These includes CDC, WHO, and Johns Hopkins. The Kenyan guidelines have adopted the WHO staging system (24).
1.4 HIV THERAPY.

The introduction of highly active anti-retroviral therapy (HAART) in 1996 revolutionized the care of patients with HIV/AIDS. Though not curative, these drugs suppress viral replication thus preventing further disease progression and immune system damage. Five classes of anti-retroviral drugs are currently in use worldwide. These include:

1) Nucleoside reverse transcriptase inhibitors (NRTI)
2) Non - Nucleoside reverse transcriptase inhibitors (NNRTI)
3) Protease inhibitors (PI)
4) Fusion inhibitors.
5) Integrate inhibitors

The preferred regimen consists of two nucleoside analogs combined with either a boosted PI or NNRTI or with distinct restrictions, a third nucleoside analog (25). National guidelines in Kenya recommends the use of Lamivudine, Stavudine and either Nevirapine Or Efavirenz as first line while Didanosine, Abacavir plus Lopinavir/Ritonavir (kaletra,aluvia) or Tenofovir, Abacavir plus Lopinavir/Ritonavir being reserved for second line HAART(22). There is no gold standard in terms of regimens currently in use as none has been shown to be superior to the others (26-28). In the 2NN study, the efficacy of Nevirapine was compared to Effavirenz with a Stavudine and Lamivudine backbone. There was no statistically significant difference between the two regimens in the control of viraemia at 48 weeks (28). Therefore, these two drugs can be used interchangeably in situations where one or the other is not well tolerated or cannot be used with good results (27).

Access to Anti-Retroviral therapy for HIV infected patients in the developing world is a global health priority. With the support of multi-lateral and bilateral programmes, non-governmental organizations, and national authorities, WHO had ambitious objective of treating 3 million people by the year 2005. Generic formulations were widely regarded as being critical in scaling up AIDS treatment in developing countries if this goal was to be realized since Generic drugs are much cheaper than branded formulations. However it took quite a while prior to
these drugs being recommended by some of the major donor agencies such as the USA's multi-billion dollar PEPFA programmed even though they have FDA approval (29).

In addition to political considerations, particularly on the legitimacy and the consequences of using generic instead of branded drugs, this situation was partly explained by the absence of clinical studies showing the efficacy and tolerability of generic drugs (30). However, in a study carried out by Christian Laurent and colleagues, looking at the efficacy of fixed-dose combination of Stavudine, Lamivudine and nevirapine, they found these drugs to be as efficacious as the branded drugs in suppressing viral loads to un-detectable levels at 24 weeks. (80% of the study group had un-detectable viral load) and significant increase in the CD4+ counts within the same periods (31). These findings lend the much-needed support to the generic fixed-dose combination of above drugs. Kenya, like most third world countries is currently using mainly generic formulations in its health facilities.

1.5 IMMUNOLOGICAL RESPONSE IN PATIENTS ON HAART.

Following the introduction of HAART, the natural history of HIV infection changed to an un-natural one conditioned by success and/or failure of treatment (33-35). Current goals of effective ART therapy are to maintain health and well-being with minimal side effects for as long as possible. This is achieved by suppressing the viral load to below the limit of detection for as long as possible, which leads to preservation or restoration of the immunological function, even though discordant responses may occur. The ultimate effect of all these processes is the improvement of quality of life and reduction of HIV related morbidity and mortality (36). Effective HAART that durably suppresses HIV replication results in a sustained rise in the absolute CD4+ count. This increase has been observed to occur in two phases: an initial rapid rise in the first few months primarily due to redistribution of CD45Ro memory cells previously sequestered in lymphoid
tissues and generalized reduction in apoptotic cell death (37). This is followed by a slower second phase of CD4+ cell expansion that persists for years with suppressive anti-retroviral therapy. This observation represents expansion of naïve CD45RA cells as thymic function is restored and results in long-term sustained rise in CD4+ cell count. The sustained increase in CD45RA correlates with the magnitude of viral load suppression and its stability over time (38). To this effect, studies have shown that the amount of thymic tissue present at initiation of HAART correlates well with the magnitude of subsequent increase in naïve T cells (39). A prospective study by Smith CJ et al, looking at a cohort of 237 patients on HAART with baseline median CD4+ count of 175 x 10^6 cells/l and viral suppression maintained for one year, found that there was more sustained increase in CD4+ T-cell count. After an initial rapid rise in median CD4+ cell count of 97.2 x 10^6 /l in the first month, the rate of CD4+ increase subsequently diminished with rates of 11.6 x 10^6 /l from year one to year two and an estimated rate of 5.4 x 10^6 /l/month at year two (40). A more recently published study (Smith K et al) with a six-year follow up of patients found that CD4+ cell count continued to increase, albeit slowly during year three to six of HAART (41). A study by Lawn SD and Linda G demonstrated an absolute increase in CD4+ count of 200-400 cells/mm^3 after two to four years of HAART (36). The CD4+ is generally a mirror image of the HIV RNA decay curve, with increases that average 50-60 cells/mm^3 in the first four months with subsequent increases of 3-6 cells/mm^3/month or 50-100 cells/mm^3/year with good viral suppression (36). Fernando and Santiago while looking at long-term outcomes among patients infected with HIV after successful viral suppression followed up 225 patients for twenty-four months. After this duration they found out that 16.5% of patients had a median CD4+ cell count increase of less than 100 x 10^6/mm^3 whereas 83.5% had achieved a median CD4+ count of more than 100 x 10^6 cells/mm^3 (42). Grabber S et al looked at immunological and virological response among patients on HAART after six months of treatment. They defined good response as CD4+ change of more than 50 cells/ml at six months of therapy. Two thousand two hundred and thirty-six patients were followed up in this study and ART naïve
patients were put on a PI based regimen. 47.5 % of the patients recorded both good immunological and virological responses, 19% had good immunological response with poor virological response, and 16.2% had both virological and immunological poor responses while 17.3% had a poor virological response. Therefore 66.5% of the study group had good immunological response while 33.5% registered a poor response. In this study, they also demonstrated that immunological response at six months of HAART indicates a favorable clinical response in AIDS patients regardless of virological response. (43). In a similar study by Kilaru KR (44) and colleagues, 116 patients with a median base line CD4+ cell count of 75 cells/mm$^3$ were evaluated. After six months of therapy, this rose to 114cells/mm$^3$. This study documented good immunological response in 79.1% of the subjects defined as a change more than 50 cells/mm$^3$. They thus recorded a good response at six months inspite of low baseline counts in most patients. Baseline CD4+ count was not a factor in achieving good immunological response at six months (44).

While viral suppression is a key determinant of long-term CD4+ cell recovery (45), the nadir CD4+ count at the time of HAART initiation may also be an important factor (46-50). Smith et al looking at factors influencing increase in CD4+ cell count in patients receiving long term HAART therapy recorded a median increase of 114cells/mm$^3$ after six months of treatment with higher values at 12 and 24 months respectively. They found that the lower baseline CD4+ cell count was associated with greater CD4+ cell count increase in the first three months while the cumulative proportion of time spent with viral load of less than 400 copies/ml was associated with a more favorable change in CD4+ count there after (51). A study done by Barl P A et al, found that there was CD4+ cell repletion in both blood and lymphoid compartments within the first year of HAART among patients with early disease and baseline CD4+ cell count above 400x10$^6$cells/l (50). Similarly, Lederman and colleagues found that CD4+ cell numbers had not normalized after nearly three years of follow up among patients with severely depleted CD4+ cell count at initiation of therapy (52). Plymate and Hog in their study documented an increased risk of progressing to AIDS and...
death among patients who started HAART at CD4+ counts below 200 cells/mm$^3$ (51,53). These findings are consistent with the study done by Lawn et al in which they looked at the patients with baseline CD4+ count of less than 50 cells/mm$^3$ (54). Podzamizer D et al looked at the effect of baseline CD4+ count on immune reconstitution. They documented that lower CD4+ count is the strongest variable for not achieving CD4+ count of more than 200 cells in patients starting on HAART regimens (55).

Benefits from anti-retroviral therapy are strongly associated with the level of patient adherence. Because of high and constant rate of viral replication and mutation, it has been suggested that a level of at least 95% adherence is required in order to maintain undetectable viral levels (56-57). DAART study carried out in Mombassa-Kenya, compared two groups of patients; one group received Directly Administered Anti-retroviral Therapy (DAART) while the other group was not on active follow up. It was found that the DAART group had a higher adherence of 96% compared to 90% in the non-DAART group. Similarly, the DAART group recorded slightly higher CD4+ increase (median of 153cells/mm$^3$) as compared to 141cells/mm$^3$ in the non-DAART group (40). Since the ARV programme in this country is not based on DAART principles, we can assume that our adherence levels are 90%. This level of adherence is unacceptable for complete viral suppression and good immunological response.

Mono-therapy in management of AIDS has been associated with development of resistance (58). In South Africa and Thailand, it was demonstrated that there was emergence of NNRTI resistant variants following the use of single Nevirapine given to women in labor. 39% of the women and 42% of the infants had detectable NNRTI resistant virus in plasma six weeks after Nevirapine exposure, with K103N being the predominant mutation in mothers (59-60). Nevirapine is a key component of first line therapy in Kenya (24). It is not known what proportions of mothers who receive Nevirapine in labor eventually proceed on ART. In a study by Hassan Ali H S, primary resistance to nevirapine was reported to be 7.8% while secondary resistance was 2.9%. This high primary resistance was thought to be due to increased nevirapine therapy more so as
monotherapy in PMTCT programmes, which might have led to increased transmission of the mutant virus as opposed to the wild type. This is over and above de novo mutations of the virus (64).

Advancing age has also been implicated in poor immunologic response among patients on HAART. Thymic atrophy is touted as the likely explanation for this observation among patients over 50 years on HAART.

Gender factor in immunological response was evaluated in a study by Giordano P T et al, looking at whether, sex and race/ethnicity influence CD4+ cell response in patients who achieve virological suppression during ARV therapy. Apart from pharmacokinetic differences which tend to favor the female gender, by having higher antiretroviral drug levels in circulation thus conferring profound viral suppression with attendant CD4+ cell response, women have been found to repopulate their peripheral CD4+ cells in response to viral suppression more rapidly than men. This is occasioned by increased peripheral redistribution of memory CD4+ cells from lymphoid tissues, which is faster than that observed in men (65). This could also be explained by the fact that thymus output of naïve CD4+ cells in response to HAART, an important contributor to later CD4+ cell increase, is greater in women than in men (66-67).
2.0 STUDY JUSTIFICATION.

It is now over a decade since HAART was introduced in this country and more patients are expected to access ARV following WHO's ART for all initiative. Viral suppression with ART results in immunological response which has a bearing on clinical outcome based on assessment made at six months of therapy. However, no studies have been done in this country to establish immunological response, virological response or clinical response to antiretroviral therapy currently in use, hence the need for this study to determine immunological response to drug therapy in HIV.

A large data base on patients undergoing first line ART treatment in CCC at Kenyatta National Hospital exists since they do CD4+ count at baseline and at determined intervals regularly during follow-up period which when analyzed will yield useful hence this retrospective study.

3.0 NULL HYPOTHESIS:
There is poor immunological response in patients on first line HAART as demonstrated by insignificant rise in CD4+ cell count within the first six months of treatment.
4.1 MAIN OBJECTIVE.

To describe the early immunological response among patients on first line HAART at Kenyatta National Hospital.

4.2 SPECIFIC OBJECTIVES.

1) To document CD4+ cell count at baseline and at six months of therapy in patients on HAART.

2) To determine the proportion of patients with poor immunological response.

3) To describe the relationship between baseline CD4+ T-cell count and CD4+ cell count at six months of treatment.

4) To determine the association between demographic factors and immunological response.
DEFINITIONS

1) IMMUNOLOGIC RESPONSE.

This refers to qualitative and quantitative restoration of the immunological function, mainly CD 4 + T cells, which are usually depleted in HIV infection, following initiation of HAART.

For the purposes of this study, this was limited to quantitative response of CD 4 + cells within the first six months of treatment.

Poor immunological response in this study refers to a change in CD4+ T-cell count of less than 50 cells/ mm³ from baseline after six months of therapy while good response denotes a change of more than 50 cells/ mm³. (43)

2) HAART

Defined as triple therapy consisting of nucleoside analog backbone and NNRTI or PI's

3) FIRST LINE HAART

This refers to a regimen comprising of Stavudine and Lamivudine as a backbone plus either nevirapine or efavirenz. (2)
5.0 METHODOLOGY

5.1 STUDY DESIGN. This was a retrospective analytical study evaluating immunological response among HIV infected patients following six months of treatment with HAART at Kenyatta National Hospital's Comprehensive Care Clinic.

5.2 STUDY POPULATION. Study population consisted of HIV positive patients seen at the comprehensive care clinic in Kenyatta National Hospital, who had been on first line HAART therapy for six months.

5.3 INCLUSION CRITERIA. The study included all adult (15 years and above) patients who were on first line therapy for six months by June 2007 and who had baseline CD4+ cell count done before commencing therapy and repeat counts at six months of treatment.

5.4 EXCLUSION CRITERIA. The study excluded all patients who had co-morbidities that would influence immune function such as diabetes mellitus, autoimmune disorders, malignancies and active Tuberculosis during the first six months of treatment. Also excluded were patients using any other ARV regimen not defined in this study as first line.

5.5 SAMPLE SIZE

This was a population survey study whereby all patients on treatment within the stipulated period and meeting the inclusion criteria were recruited. However, a minimum sample size of 382 was required for significant statistical analysis. This number was arrived at using the calculation formula for prevalence study.
\[ N = \frac{z^2 p(1-p)}{d^2} \]

where,

\[ z = 1.96 \]

\[ p = \text{prevalence from a study by Grabber et al which recorded good response in 45.6} \% \text{ of the study population (43).} \]

\[ d = \text{confidence interval of 95\%} \]

5.6 DATA COLLECTION

A list of patients on antiretroviral therapy from 2003 to June 2007, was obtained from the CCC pharmacy where all patients’ information is computerized. Files for patients on first line therapy were selected and reviewed and those meeting the exclusion criteria excluded from the study. From eligible files, the following information was extracted:

Demographic data, which included age, sex, marital status, educational background at initiation of therapy;

Patient’s WHO clinical stage at the initiation of therapy;

CD4+ cell count at initiation of therapy and at six months of treatment and adherence level as recorded in the file.

Data collection was done by the principal investigator.

5.7 OUTCOME VARIABLE

Outcome variable for this study was CD4+ change after six months of therapy.
5.8 DATA MANAGEMENT

Relevant information was collected using a data profoma and later transferred onto a data entry sheet. It was then cleaned and verified to ensure quality maintenance. Analysis was performed using Statistical Package for Social Sciences version 15 software for windows. Change in CD4+ cell count was calculated from the formula:

\[ A = C - B \]

Where

- **A** = immunological response
- **B** = baseline CD4+ cell count
- **C** = CD4+ cell count after six months of therapy.

Both Descriptive statistics and inferential statistics were done. Descriptive statistics for categorical variables were frequency distributions and proportions while measures of central tendency and dispersion were used for continuous variables. Inferential statistics included the chi-square test, which was used to determine associations between categorical variables, where a p value of less than 0.05 was deemed significant. Linear regression was performed to establish the association between baseline CD4+ cell count and CD4+ cell count after six months of therapy. Proportion of patients achieving good immunological response at six months was determined.

ETHICAL CONSIDERATION

Approval for this study was obtained from the department of internal medicine and Kenyatta National Hospital's Ethics and Research committee. Authority to review files was obtained from the Deputy Director (clinical services), KNH. Findings of this study will be communicated to comprehensive care clinic to facilitate decision-making in patient management.
6.0 RESULTS

FIG 1: Flow Chart for Selection of Study Participants

One thousand six hundred and seventy-eight files of patients on first line HAART were reviewed with 800 patients satisfying the inclusion criteria for the study. The rest of the files were excluded mainly due to lack of repeat CD4+ cell count at six months and co-morbidities that included diabetes mellitus, tuberculosis and malignancies.
### 6.1 BASELINE CHARACTERISTICS

**TABLE 1: Baseline Demographic Characteristics of the Study Participants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n = 800)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>327 (40.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>473 (59.1%)</td>
</tr>
<tr>
<td>M:F ratio</td>
<td>1:1.4</td>
</tr>
<tr>
<td>Age</td>
<td>Range 20 – 71 years</td>
</tr>
<tr>
<td>Mean</td>
<td>38.1 years</td>
</tr>
<tr>
<td>Median</td>
<td>37 years</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>144 (18%)</td>
</tr>
<tr>
<td>Married</td>
<td>513 (64.1%)</td>
</tr>
<tr>
<td>Separated</td>
<td>87 (10.9%)</td>
</tr>
<tr>
<td>Widowed</td>
<td>56 (7%)</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>18 (2.3%)</td>
</tr>
<tr>
<td>Primary</td>
<td>242 (30.3%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>450 (56.3%)</td>
</tr>
<tr>
<td>College</td>
<td>56 (7.0%)</td>
</tr>
<tr>
<td>University</td>
<td>34 (4.3%)</td>
</tr>
</tbody>
</table>

Male to female ratio in this study was 1:1.4 with majority of the participants being married and small proportion of the subjects lacking formal education. Mean age for the group was 38.1 years with a median of 37 years. Most of our study subjects had attained formal education with majority being high school graduates. Similarly, most of the patients were married. Demographic characteristics of the excluded patients were similar to the study cohort hence their exclusion did not necessarily introduce a bias in this study.
Majority of patients with HIV on ART are young adults between 30-44 years of age. There was Female dominance in Male to female ratio in all age groups below 44 years with a reversal in patients aged 45 years and above. This change was however statistically insignificant.
6.1.2 WHO STAGE OF THE STUDY SUBJECTS AT TREATMENT INITIATION.

FIG. 3: Baseline WHO stage of the study subjects

Majority of patients (78%) in stage III and IV with stage III accounting for more than half of the study population (58%).
Most patients had low baseline CD4+ T-cell count with 76.3% being full-blown AIDS by definition. Mean baseline CD4+ T-cell count was 127.2 cells /mm$^3$ with median count of 116.50 cells /mm$^3$ and a range of 1-605 cells /mm$^3$. 
6.2 CD4+ CELL COUNT AT SIX MONTHS

The overall mean CD4+ cell count at six months of treatment was 239.81 cells /mm$^3$ with median count of 218.0 cells /mm$^3$ and standard deviation of 130.491. The range was 6-1053 cells/mm$^3$. Compared to males, female had higher CD4+ cell counts after six months of therapy (mean count of 243.69 cells/mm$^3$ with standard deviation of 112.2 cells/mm$^3$) compared to their male counterparts (mean count of 217.9 cells/mm$^3$). However, this was statistically insignificant with p value of 0.599 on test for equal variance.
6.3 CD4+ CHANGE AT SIX MONTHS OF HAART:

FIG. 6: CD4+ change at Six Months of HAART

In total, 748 patients representing 93.5% of the study population registered an increase in CD4+ cell count after six months of HAART with only 6.4% registering a decline in their CD4+ counts after a similar duration. The proportion that attained an increase in CD4+ cell count was 93% while men recorded an increase in 94.2%. Likewise, 6.8% of females had a decrease in CD4+ cell count compared to 5.8% for males. However, there was no statistically significant difference in response between males and females (p value of 0.699).
6.4 CD4+ CHANGE IN PATIENTS WITH POSITIVE RESPONSE

FIG. 7: CD4+ Change In Patients With positive Response.

For patients with an increase in CD4+ cell count at six months, their Mean Baseline CD4+ count was 115.66 cells/mm$^3$, and 232.91 cells/mm$^3$ at six months of HAART. Overall, mean CD4+ change was 117.246 cells /mm$^3$. 
6.5 IMMUNOLOGICAL RESPONSE.

Table 2: Immunological response after six months of HAART

<table>
<thead>
<tr>
<th>Immunological response</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>70 (21.4%)</td>
<td>109 (23%)</td>
<td>179 (22.4%)</td>
</tr>
<tr>
<td>Good</td>
<td>257 (78.6%)</td>
<td>364 (77%)</td>
<td>621 (77.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>327 (100%)</td>
<td>473 (100%)</td>
<td>800 (100%)</td>
</tr>
</tbody>
</table>

77.6% of the study subjects had good immunological response while 22.4% registered poor response. Males had better response than females even though this was statistically insignificant (p value 0.587).

6.6 ASSOCIATION BETWEEN DEMOGRAPHIC FACTORS AND IMMUNOLOGICAL RESPONSE.

6.6.1 SEX

Male subjects registered a slightly better immunological response than females (78.6% Vs. 77%). However, this was not statistically significant with a p value of 0.586 (Table 2).
Good immunological response of 72.5% to 80.5% was observed in all age groups with the lowest value being recorded in the 51 to 60 age groups. This age group also recorded the highest frequency of poor response. However, this observed difference was statistically insignificant with a p value of 0.345. Hence, there was no relationship between age and immunological response.
Mean baseline CD4+ cell count was 127 cells/mm³ while CD4+ count at six months of therapy was 239.8 cells/mm³ giving an overall mean CD4+ cell count change of 112.8 cells/mm³. CD4+ cell count at six months, hence, CD4+ cell count change was dependent on baseline CD4+ cell count. Analysis of variance (ANOVA) with six-month CD4+ count as the dependent variable was significant with a value of < 0.0001 (table 3 below).
**TABLE 3: ANOVA FOR CD4+ DIFFERENCE WITH CD4+ CELL COUNT AT SIX MONTH AS THE DEPENDENT VARIABLE AND BASELINE CD4+ COUNT AS A CONSTANT.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>7129621.117</td>
<td>1</td>
<td>7129621.117</td>
<td>878.576</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>6475745.852</td>
<td>799</td>
<td>8114.970</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13605366.969</td>
<td>800</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Baseline CD4+ cell count was a predictor of CD4+ cell difference after six months of treatment by ANOVA, with significance of <0.0001.
RELATIONSHIP BETWEEN BASELINE CD4+ CELL COUNT AND CD4+ COUNT AT SIX MONTHS.

FIG 10: Linear Regression of Baseline CD4+ Count and CD4+ Count At Six Months.

Linear regression performed to establish the relationship between baseline CD4+ count and CD4+ count at six months showed that baseline CD4+ count was predictive of the CD4+ cell count after six months of treatment. (CD4+ after six months = 114.90 + 1.02(baseline CD4+ count); R-square=0.62 at 95% mean prediction index).
8.0 DISCUSSION

HIV/AIDS epidemic in this country has taken its toll on the productive age group with most of the affected people being in the fifteen to forty nine years age bracket (12). Our study had a young population with mean age of 38.1 years and median age of 37 years (table 1). This compares well with other studies in this area with Kilaru et al (44) reporting a mean age of 37 years. Even though our age distribution mirrors National demographic statistics on HIV/AIDS, patients below twenty-five years of age accounted for only 2.9% of patients on first line HAART at Kenyatta National Hospital’s CCC. Given that we have many health facilities in Nairobi offering HIV/AIDS services besides KNH, one would sincerely hope that the low figures reflect low preference for KNH as a site and not failure of accessing therapy for those deserving patients. No study has profiled HIV staging of this particular age group in this country hence it is not possible to ascertain the proportion of patients who are currently in need of ART in this age cluster.

In this country, HIV/AIDS is more prevalent among women than in men with a male to female ratio of 1:2 (12). This study had more women than males accessing first line Anti-Retroviral therapy with male to female ratio of 1:1.4. Compared to the national statistics, this ratio implies that slightly lower proportion of women was receiving ART in our centre compared to the proportion of males. Health seeking behavior differences as well as facility preference maybe has a role in this observed difference.

According to centre for disease control (CDC) staging system, any HIV infected patient with a CD4+ cell count of less than 200 cells/ mm$^3$ is considered to have AIDS (70). Seventy-six point three percent (76.3%) of patients in our study had baseline CD4+ cell count of less than 200 cells/ mm$^3$ with 44.6% of the patients having CD4+ cell counts of less than 100 cells/mm$^3$ (fig4). The mean CD4+ cell count was 127.2 cells/ mm$^3$ and most of our patients were in WHO stage III and
Looking at the WHO stage of our patients, late presentation to CCC could have played a major role in the observed low baseline CD4+ cell counts. It is also possible that the entry point to CCC for most patients could have been an opportunistic ailment that necessitated an evaluation of the immunological status hence commencement on ART.

The observed low baseline CD4+ cell count can also be explained by the National guidelines for ARV therapy in Kenya prior to 2008, which recommended initiation of therapy in patients with CD4+ counts of less than 200 cells/mm³.

Beaurocracy in our referral system may also play a role in delaying patients from accessing ART on time. This study recruited patients who started on ART from as early as 2003. It is possible that most of these patients could have been inherited from the dark era in the history of HIV management when only the rich had access to treatment thus the poor presenting late for treatment when HIV care became accessible to all.

Low baseline CD4+ count was reported in similar work done by Kilaru et al (44) and Grabber et al (43). Kilaru et al had a mean baseline CD4+ count of 75 cells/mm³ while Grabber et al recorded a mean baseline CD4+ count of 74 cells/mm³. However, these findings need to be interpreted on the background of differences in study design whereby Kilaru’s study was specifically designed to evaluate immunological response among patients with low CD4 counts.

Adequate viral suppression results in immunological response characterized by an increase in CD4+ cell count and improved function of these cells that leads to improved quality of life by reduction of opportunistic infections. In this study, 93.5% of the study subjects registered a positive increase in CD4+ cell count at six months with a mean CD4+ change of 117.254 cells/mm³ (baseline mean count of 115.66 cells/mm³ and 232.91 cells/mm³ at six months) (fig. 7). However, good immunological response as defined by CD4+ change of 50 cells/mm³ at six months of therapy, was observed in 77.6% of the study subjects. This change was registered in spite of low baseline CD4+ count for the study group. This was higher than what was found by Grabber et al (43) even though their study had a
bigger sample size than ours and patients received a PI based regimen whereas our patients were on NNRTI based regimen. Kilaru et al (44) had slightly better results at 79.1% than what we found. However, we had a larger sample size than his (800 vs. 116) and he too used a PI based regimen.

Good adherence to ART is a prerequisite to sustained viral suppression and hence good immunological response. As shown by DAART Study (56), patients using directly administered ART strategy (DAART arm) had better response attributed to high level of adherence than the non-DAART arm. Our study did not assess adherence even though the high levels of immunological response may be attributed to probable good adherence practices among our study cohort. A study aimed at evaluating adherence levels within CCC will be useful to establish this position.

Currently Kenya has over one million people living with HIV/AIDS. The findings from this study are quite encouraging as more than 776000 people can be expected to register good immunological response to first line HAART, were they to be started on first line ARV today, thus reducing morbidity burden that this lot poses to the health-care sector. The fact that the immunological response is not dependent on age and sex is yet another plus for our population where the infection cuts across the board in terms of age and sex. However the finding that CD4+ cell count level at six months is dependent on baseline CD4+ cell count implies that most of our patients may require slightly longer duration before attaining CD4+ values that are associated with low incidence of opportunistic infections. Since they tend to present with very low baseline CD4+ counts.

Patients accessing ART at KNH are mainly on generic formulations made available through the Government of Kenya initiative supplemented by partners such as PEPFAR and Clinton Foundation. Good immunological response recorded in this study compares well with studies done using branded drugs (31), hence, pointing towards good efficacy of generic drugs thus lending support to ART programmes in the third world countries. Christian L et al did a study in which they evaluated the efficacy of generic formulations compared to branded drugs (lamivudine, stavudine and nevirapine). In their results, they registered no
statistical difference in these two groups of patients in terms of virological response and CD4+ cell count increase (31). This is quite important more so for Sub-Saharan countries which are not well endowed with finances yet they shoulder the heaviest burden of HIV/AIDS World-wide (5).

Poor immunological response occurred in 22.4% of our study patients. This is slightly higher than that reported by Kilaru et al (44) but much lower in comparison to Grabbers (43). This poor response seems not to have any association with baseline CD4+ count, age or sex. Gliola et al (68) when looking at immunological and virological response to HAART therapy in a community-based cohort of HIV positive individuals found out that treatment history, and initial virological response but not baseline CD4+ cell count, were associated with the probability of having poor immunological response. Our study did not look at virological response, but going by above findings, poor virological response might be a key factor in this group of poor responders immunologically. The role of virological response in this finding, a study carried out by Hassan Ali (69) in Kenyatta National Hospital, documented primary phenotypic resistance of 25.5% and secondary resistance of 4.5% to Nevirapine, a drug that is commonly employed in our first line regimens. Thus, primary resistance to nevirapine might contribute to the poor response seen in this study. It is therefore prudent to carry out periodic phenotypic or genotypic resistance testing in these patients to establish the actual position.

Opportunistic infections in HIV/AIDS has been noted to occur most frequently when the absolute CD4+ count falls below 200 cells/mm$^3$. In this study 44.7% of the subjects were not able to attain an absolute CD4+ count of 200 cells/mm$^3$ by six months of therapy, with those having more than 200 cells/mm$^3$ being just slightly over half (55.3%). Thus, quite a significant proportion of our patients are still at risk of opportunistic infections despite the fact that they have been commenced on HAART. This poor response was associated with low base line CD4+ cell count which was further illustrated on linear regression which showed significant relationship between baseline and CD4+ count at six months of therapy (CD4+ count at six months = 114.90 + 1.02(baseline CD4+ count); 95%
mean prediction index =0.62 ) (fig 10). Patients with very low CD4+ count may not be able to mount a rapid immunological response characterized by redistribution of CD45Ro memory cells as well as the slow increase that represents expansion of naïve CD45RA cells as thymic function is restored.

9.0 CONCLUSION
This study demonstrated good immunological response among patients on first line HAART in Kenyatta National Hospital. Therefore, first line HAART regimen currently in use (stavudine, lamivudine and nevirapine or efavirenz) is effective if immunological response alone is considered. This study has also demonstrated the utility of Baseline CD4+ count in predicting CD4+ T cell count at six months of therapy. Our findings compared well with other studies that evaluated this subject.
10.0 RECOMMENDATION

1). First line antiretroviral therapy regimen as currently employed in our comprehensive care clinic is effective based on immunological response. We therefore recommend this regimen to be maintained.

2). Antiretroviral therapy should be initiated early before baseline CD4+ cell count falls too low as this has a bearing on CD4+ count at six months. However, Comparative study should be carried out in order to determine the best level of baseline CD4+ cell count at which therapy should be initiated.

11.0 STUDY LIMITATIONS

1). Viral load as a test is not routinely done in CCC hence virological response was not assessed in this study.

2). Drug to drug interactions and their side effects were not evaluated in this study.

3). We excluded patients with co-morbidities hence above recommendations may not necessarily apply to them.

4). Adherence was not assessed in this study.

5). We did not document co-morbid conditions within the six months interval especially in ambulatory patients. Thus, immunological response might be higher than that recorded in this study.

6). A significant proportion of files were excluded from the study due to missing data due to retrospective nature of the study.
12.0 REFERENCES


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APPENDIX 1

Revised World Health Organization (WHO) Clinical Staging of HIV/AIDS for Adults and Adolescents (2005)

(This is the interim African Region version for persons aged 15 years or more who have had a positive HIV antibody test or other laboratory evidence of HIV infection) (It must be noted that the UN defines adolescents as persons aged 10–19 years but for surveillance purposes, the category of adults and adolescents comprises people aged 15 years and over)

Primary HIV infection

• Asymptomatic
• Acute retroviral syndrome

Clinical stage 1

• Asymptomatic
• Persistent generalized lymphadenopathy

Clinical stage 2

• Moderate and unexplained weight loss (<10% of presumed or measured body weight)
• Recurrent respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis)
• Herpes zoster
• Recurrent oral ulcerations
• Papular pruritic eruptions
• Angular cheilitis
• Seborrhoeic dermatitis
• Fungal finger nail infections

Clinical stage 3

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

• Unexplained chronic diarrhoea for longer than one month
• Unexplained persistent fever (intermittent or constant for longer than one month)
• Severe weight loss (>10% of presumed or measured body weight)
• Oral candidiasis
• Oral hairy leukoplakia
- Pulmonary tuberculosis (TB) diagnosed in last two years
- Severe presumed bacterial infections (e.g. pneumonia, empyema, meningitis, bacteraemia, pyomyositis, bone or joint infection)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary

- Unexplained anaemia (< 80 g/l), and or neutropenia (<500/µl) and or thrombocytopenia (<50 000/ µl) for more than one month

Clinical stage 4

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month’s duration)
- Oesophageal candidiasis
- Extrapulmonary Tuberculosis
- Kaposi’s sarcoma
- Central nervous system toxoplasmosis
- HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary

- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Candida of trachea, bronchi or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
- Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
- Recurrent non-typhoidal salmonella septicaemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Visceral leishmaniasis
APPENDIX 2

DATA PROFORMA

Serial number ____________________________________________

Age _____________________________________________________

Sex \[ M [ ] \] \[ S [ ] \]

Marital status \[ S [ ] \] \[ M [ ] \]

Educational standard \[ P [ ] \] \[ S [ ] \] \[ C [ ] \] \[ U [ ] \]

WHO clinical stage \[ I [ ] \] \[ II [ ] \] \[ III [ ] \] \[ IV [ ] \]

Cormobid conditions _______________________________________

Baseline CD4+ cell count ________________________________

CD4+ cell count at six months ____________________________

Level of adherence _____________________________________