**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,KUMASI**

**SCHOOL OF GRADUATE STUDIES**

**SCHOOL OF MEDICAL SCIENCE**

**DEPARTMENT OF CLINICAL MICROBIOLOGY**

**TITLE:**

# EFFECT OF HIV-2 CO-INFECTION ON HIV 1& 2 DUALLY INFECTED PATIENTS' RESPONSE HAART

**A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE DEGREE (MSC) IN CLINICAL MICROBIOLOGY**

**BY**

**ALBERT DOMPREH**

**JULY 2008**

## DECLARATION

I hereby declare that this submission is my own work toward the MSc (Clinical Microbiology) degree and that to the best of my knowledge, it contains no material previous published by another person or material which has been accepted for the award of any other degree of the university except where due acknowledgement has been made from the text.

ALBERT DOMPREH Signature…….

Date…….

(STUDENT)



DR. T.B KWOFIE Signature…….

Date…….

(SUPERVISOR)

PROF. YAW ADU-SARKODIE Signature…….

Date…….

(HEAD OF DEPARTMENT)

## ACKNOWLEDGEMENT

Glory be to the Lord for the successfully completion of this work.

I acknowledge sincerely the support given to me by my supervisor Dr. T. B. Kwofie, a senior lecturer of the Department of Clinical Microbiology, KNUST. I appreciate his tolerance, useful suggestions and his words of encouragement.

I am most grateful to Abigail Ameyaw of the Public Health Unit and Sister Aisha Yussif, a senior nursing officer at the HIV Clinic of the Komfo Anokye Teaching hospital.

Many thanks also to Dr. Nguah Blay from the Department of Child Health for helping with the statistical analysis. His contribution is highly appreciated.

I appreciate the moral support from my good friends Isaac Boakye of the Research and Development Unit – KATH and Lawrence Annison, an MPhil student from the Department of Clinical Microbiology – KNUST. They showed a lot of interest in this work and could not wait to see the work completed.

Finally, to all who in one way or the other contributed to the success of this work, I say thank you and stay blessed.

## TABLE OF CONTENT

## LIST OF FIGURES

**FIGURE** **PAGE**

## LIST OF TABLES

**TABLE** **PAGE**





## LIST OF ACRONYMS

| | | |
|---|---|---|
| HIV | - | Human Immunodeficiency Virus |
| AIDS | - | Acquired Immunodeficiency Syndrome |
| LAV | - | Lymphadenopathy Virus |
| HTLV | - | Human T-Lymphotropic Virus |
| SIV | - | Simian Immunodeficiency Virus |
| OPV | - | Oral Polio Vaccine |
| VCT | - | Voluntary Counselling and Testing |
| PMTCT | - | Prevention of Mother to Child Transmission |
| ART | - | Antiretroviral Therapy |
| HAART | - | Highly Active Antiretroviral Therapy |
| KATH | - | Komfo Anokye Teaching Hospital |
| NRTI | - | Nucleoside Reverse Transcriptase Inhibitor |
| NNRTI | - | Non-Nucleoside Reverse Transcriptase Inhibitor |
| PI | - | Protease Inhibitor |
| NACP | - | National AIDS Control Programme |

| | | |
|---|---|---|
| DNA | - | Deoxyribonucleic Acid |
| RNA | - | Ribonucleic Acid |
| FHI | - | Family Health International |
| CD4 | - | Cluster of Differentiation |
| ELISA | - | Enzyme Linked Immunosorbent Assay |
| TMB | - | Tetramethylbenzedine |
| HPR | - | Horseradish Peroxidase |
| PCR | - | Polymerase Chain Reaction |
| CMV | - | Cytomegalo Virus |
| TB | - | Tuberculosis |
| WHO | - | World Health Organization |
| MOH | - | Ministry of Health |
| GHS | - | Ghana Health Services |
| OD | - | Optical Density |
| CO | - | Cut-off |

**ABSTRACT**

This study was carried out to determine the effect of HIV-2 on HIV-1 & 2 dually infected patients' response to antiretroviral therapy supposedly designed for HIV-1. HIV-1 and HIV-2 are distinct strains of viruses that cause AIDS. HIV-2 is known to be less pathogenic with a slower rate of CD4 depletion and a longer time of disease progression to AIDS. In contrast, HIV-1 is more pathogenic, depletes CD4 faster with a comparatively shorter time disease progression to AIDS. HIV-1 and HIV-2 differ in their nucleotide sequence and their genetic organization with just about 30% homology between them. Therefore, one would expect that their replicative steps in an infected individual could possibly involve different enzymes and cellular factors. Available antiretroviral drugs target only HIV-1 and act by interfering with the replicative steps of the HIV virus. In line with this, a drug supposedly designed for HIV-1 might not provide enough inhibition for HIV-2. We specifically monitored the response of patients undergoing ART with HIV-2 alone infection and HIV -1 & 2 dual infections and compared their response to patients with HIV-1. In all, 108 patients were enrolled into the study. Out of this, 87% had HIV-1, 7% had HIV-2 while 6% had HIV-1 & 2. The patients were monitored using their CD4 kinetics over a period of two years with a baseline CD4 and four consecutive counts performed at 6 months intervals. The results indicated that patients with HIV-2 infection responded favorably to the therapy just like HIV-1 infected patients. However, it appears that the synergistic effect of HIV-1 & HIV- 2 in HIV-1 & 2 dual infections contributed to a comparatively lower response to therapy. It was also found that, most probably, the modes of action of the drugs are on host factors but not virus specific factors. Not withstanding the difference in viral genome, HIV-2 patients responded well to the supposed HIV-1 specifically designed drugs.

# CHAPTER ONE

## Introduction

The Human Immuno Defficiency Viruses (HIV) type-1 and type-2 are two distinct strains of human lentiviruses of the Retrovirinae family that cause AIDS (Clavel et al, 1987). HIV-1 is the predominant strain with a worldwide distribution but HIV-2 is mostly confined to West Africa (Fischetti et al, 2004).

According to Kanki, HIV-2 is less pathogenic, has a slower rate of CD4 depletion and a longer time of disease progression to AIDS (Kanki JP, 1999). In contrast, HIV-1 is more virulent, depletes CD4 cells faster with comparatively shorter time disease progression to AIDS. In line with this observation, HIV-1 infections have constantly higher viral load as compared to HIV-2 infections (Simon F et al, 1993). Therefore, one would expect that patients infected with HIV-1 would progress to AIDS faster than patients with HIV-2 due to the pathogenic differences between the two viruses, thus HIV-1 being more pathogenic.

However, Whittle and coworkers in 1999 observed that the mortality rate for dual infections was similar to that of HIV-1 infections. Meanwhile, dual infections with the two viruses would either lead to a possible synergistic or complementation of the two viruses, resulting in an accelerated progression to AIDS due to increasing viral burden of the two HIV's than infections with only one of them. Or a possible antagonistic or interference of the two viruses may lead to slower progression to AIDS. So the study of Whittle and coworkers may suggest that infection with the two HIV's do not complement nor interfere with each other as mortality rate was the same in both HIV-1 and HIV-1/2 dually infected patients. However, this was a study done

in relation to mortality rates of individuals but not in relation to the patient's response to therapy. HIV therapy, otherwise known as Highly Active Antiretroviral Therapy (HAART) acts by interfering with the replication steps of the virus in the individual host. Since HIV-1 and HIV-2 differ in their nucleotide sequences, and for that matter, their genetic organization, one would expect that their replication steps in an infected person could possibly involve different enzymes and cellular factors. Therefore a drug supposedly designed for HIV-1 might not provide adequate inhibition for HIV-2. There appear not to be enough literature available in this respect.



This study was therefore undertaken to determine how HIV-2 will affect the response to patients infected with HIV-1 & 2 dual infections on HAART using the CD4 kinetics as a measure of response to therapy.

Viral load and CD4 cell count are the main markers for monitoring patients' response to HAART (Hughes et al, 1997). The measure of patients' response to HAART has not been of a generalized practice in Africa especially in Ghana. This is mainly due to lack of resources. Viral load demands a more complex, sophisticated and expensive machinery which is generally not available in resource-poor countries. CD4 cell count which is comparatively simpler and affordable has been advocated by World Health Organization as the basis for monitoring patients' response to HAART in resource-constraint areas (WHO, 2004) such as Ghana.

As part of the intervention to combat HIV/AIDS in Ghana, a number of accredited sites for HIV management have been set up. Komfo Anokye Teaching Hospital (KATH), a tertiary and a referral hospital in the country is one of such sites. The hospital is located in the Ashanti region where HIV prevalence is quite high (3.6%).

The hospital serves a lot of people over a wide geographical area. However, the hospital lacks factual data and information and there appears to be not much information available to show how people infected with either HIV-2 alone or HIV-1/2 dual infection respond to antiretroviral therapy designed specifically for HIV-1.

**Aim of Study**

The study aims at determining the effect of HIV type-2 on the response of patients with HIV-1 & 2 dual infections to therapy that has been specifically designed for HIV-1 by using their CD4 kinetics.



**Objectives**

This present work therefore seeks to specifically look at;

1. How patients infected with HIV-2 will respond to HAART.
2. The effect of HIV-2 co-infection on HIV-1/2 dually infected patients' response to HAART at KATH.
3. It was also to determine whether such response is influenced by demographic factors such as gender, age, socio-economic factors, etc.

# CHAPTER TWO

## Literature Review

### 2.0 History of HIV

Human immunodeficiency syndrome (AIDS) was first recognized in June 1981 by Gottlieb et al in Los Angeles (CDC Report, 1981). Two years later, a research team led by Professor Luc Montagnier in Paris discovered the causative agent of AIDS named it Lymphadenopathy Associated Virus (LAV) (Barre-Sinoussi et al, 1983). A year later, Professor Robert Gallo and team isolated the same virus and called it Human T-lymphotropic Virus – HTLV III (Broder et al, 1984). Each group claimed patency to the discovery resulting in a controversy but was resolved later and the virus named Human Immunodeficiency Virus (HIV).

### 2.1 Classification of HIV

HIV is a member of the genus lentivirinae of the Retroviridae family and attacks the immune system. It is an enveloped virus containing two single-stranded RNA genomes, one of which is reverse transcribed into DNA and then integrated into the human genome. The name 'lentivirus' literally means 'slow virus' because they take such a long times to produce adverse effect in the body. Once integrated in the host genome, they can not be eradicated from the host completely with any of the available antiretroviral drugs (Schiipbach J, 2003). Though HIV is human specific, counterpart virus have been found in a number of different animals, including cats, sheep, horses and cattle. However, the most interesting lentivirus in terms of the investigation into the origins of HIV is the Simian Immunodeficiency Virus (SIV) that affects monkeys.

## 2.2 The Origin of HIV

It is now generally accepted that HIV is a descendant of a Simian Immunodeficiency Virus because certain strains of SIVs bear a very close resemblance to HIV-1 and HIV-2, yet, the issue of the origin of the HIV virus still remains a puzzle. A lot of theories have come up to explain how and where the virus might have emanated from. Among these are the Hunter's Theory, the Oral Polio Vaccine Theory and the Contaminated Needle Theory (Katrak SM, 2006).

### 2.2.1 Hunter's Theory

The Hunter's Theory is the most plausible theory which alludes to the zoonotic transfer of the SIVcpz from chimpanzees to humans. The argument of this is that HIV came about as a result of chimpanzees being hunted, killed and eaten by humans or the monkey's blood getting into cuts or wounds of hunters (Moore J, 2004). The hunter's body would have fought off the SIV but on a few occasions the infected SIV adapted itself within the human host and became HIV. The fact that the many existing types of HIV show just slight differences with SIV supports the theory (Wolfe et al, 2004).

### 2.2.2 Oral Polio Vaccine Theory

The Oral Polio Vaccine (OPV) theory contends that HIV was transferred iatrogenically (i.e. via medical experiments). The production of the OPV which required growth of Polio Virus in monkey kidney cells (Carlsen, 2001) might have seen a lot of polio vaccines known as CHAT(Hooper, 1999) produced from polio virus grown on SIV-infected monkey kidney cells and this possibly contaminated the vaccine which was subsequently transferred to humans.

### 2.2.3 Contaminated Needle Theory

The Contaminated Needle Theory is an extension of the original hunter's theory. It is believed that in the 1950's disposable plastic syringes were being recycled in African healthcare settings without any proper sterilization. This was because the huge quantities of syringes needed would have been very expensive to bear (Dicko et al, 2000). It is therefore likely that viral particles must have been transferred from the hunter's blood to another, thereby creating the potential for the virus to mutate and replicate in each new individual (Marx et al, 2001).

It is likely that we will never know how, when and where HIV actually originated. Scientists investigating the possibilities often become very attached to their individual 'pet' theories and insist that theirs is the only true answer, but the spread of HIV could quite conceivably have been induced by a combination of many different events. Whether through injections, migration, wars or genetic engineering, perhaps what we should be focusing on now is not the origin of the epidemic but how we can treat those affected by it and prevent any further possible infections.

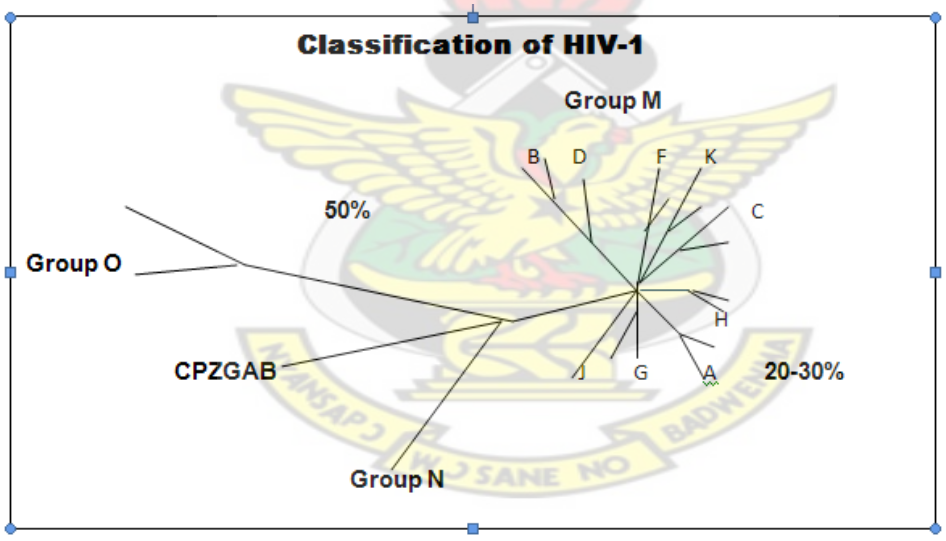
## 2.3 Types of the Virus

There are two main types of the HIV virus; HIV-1 and HIV-2, each has several subtypes. Both types belong to the retroviridae family. They are enveloped viruses containing two single-stranded RNA one of which is reverse transcribed to DNA before integration into the human genome. They are generally transmitted through sexual contact, blood transmission and vertically from mother to baby. The disease AIDS can be caused either by HIV-1 or HIV-2 or both with the disease state indistinguishable from the viral type. HIV-1 predominates all over the world but HIV-2 is mostly found in West Africa. HIV-1 has 3 genetically distinct subtypes; M,

N and O with the major group (group M) having 10 subtypes (A-J). Group O (Outliers) contains distinct group of heterogonous viruses with unevenly distribution throughout the world and the N group (Non-M, non-O) having only one known subtype which was isolated from the Republic of Cameroon. In addition to these, 14 inter-subtypes circulating recombinant forms (CRFs) of HIV-1 have been identified and documented with CRF02_AG as the predominant strain in Kumasi, Ghana (Fischetti et al, 2004). This confirmed two separate earlier reports by Cornelissen et al, 2000 and Ortiz et al, 2000 establishing the presence of CRF02_AG in Africa.



Figure 1. Classification of HIV



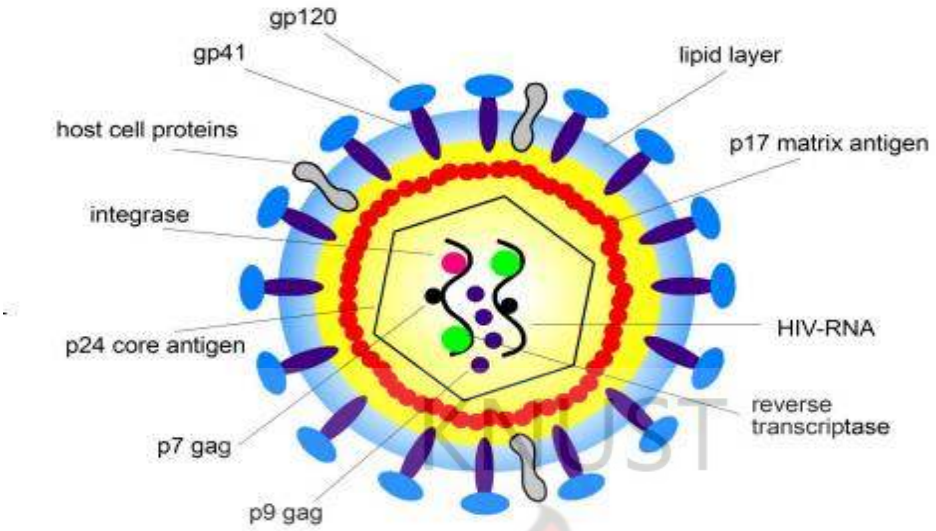

HIV type 2 is much less common originating from macaque monkeys (green monkeys) which are indigenous to western and central Africa (Requejo, 2006) especially in Senegal, Guinea Bissau, Ghana, Cote D' ivoire, Mali, Gambia and Cape Verde Island (Miyazaki, 1995). HIV-2 also has several different groups which are likely to have been derived from separate monkey species to human transmissions. It

has a comparatively less transmissible rate, slower rate of cell decline and slower level of clinical progression (Kanki, 1999).

### 2.4 Morphological Structure of HIV-1

HIV-1 and HIV-2 resemble each other under the electron microscope. However they differ in terms of the molecular weight of their proteins as well as having differences in their accessory genes. The viral particle has a diameter of 100nm with an outer lipoprotein membrane composing of 72 glycoprotein complexes, which are integrated into a lipid membrane, and are each composed of trimers of an external glycoprotein gp120 and a transmembrane spanning protein gp41. The bonding between gp120 and gp41 is a loose one. Hence gp120 can be shed spontaneously within a local environment making it possible to be detected in serum (Oh et al, 1992) and in the lymphatic tissues of the infected patients (Sunila et al, 1997). An inner lipoprotein membrane surrounds a matrix within which is the protein p17 anchored to the inner lipoprotein membrane. The p24 core antigen contains two copies of HIV-1 RNA which is part of a protein nucleic acid complex, composed of a nucleoprotein p7 and the reverse transcriptase p66 (RT), the enzymes reverse transcriptase (RT), integrase p32 and a protease p11 (Gelderblom et al, 1993).

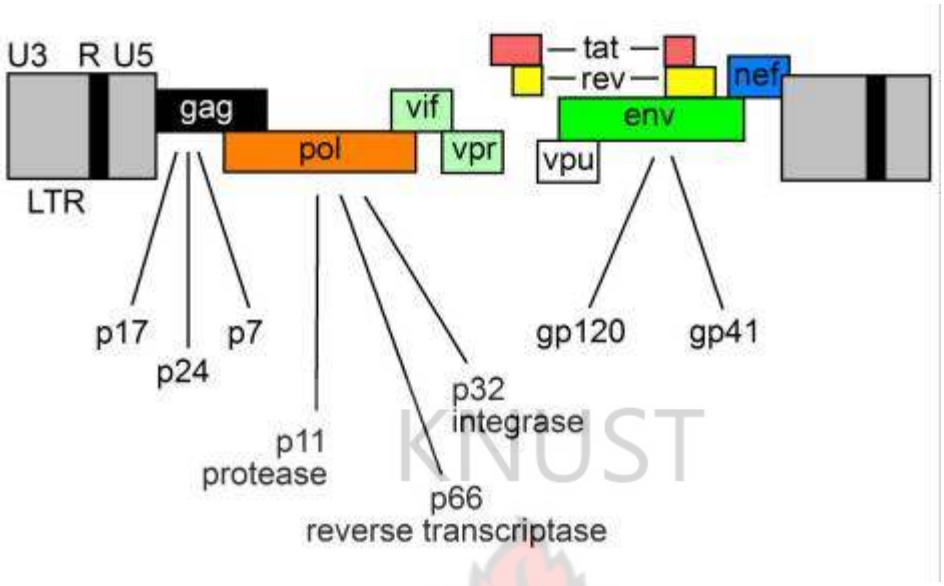
Figure 2. Structure of an HIV virion particle




## 2.5 Organization of the Viral Genome

The replication ability of the virus depends on the three genes; gag, pol and env; gag mean 'group-antigen', pol represents 'polymerase', while env stands for 'envelop' (Wong-Staal, 1991). A typical structural scheme of a retroviral genome is; 5'LTR-gag-pol-env-LTR 3'. The LTR (Long Terminal Repeat) represents the two end parts of the viral genome that are connected to the cellular DNA of the host cell after integration. The gag and the env genes code for nucleocapsid and the glycoprotein of the viral membrane but the pol gene codes for the reverse transcriptase and other enzymes. In addition HIV-1 has six other genes (vif, vpu, vpr, tat, rev and nef) in its RNA that contribute to its genetic complexity.

Figure 3. A typical structural scheme of HIV and its genes.

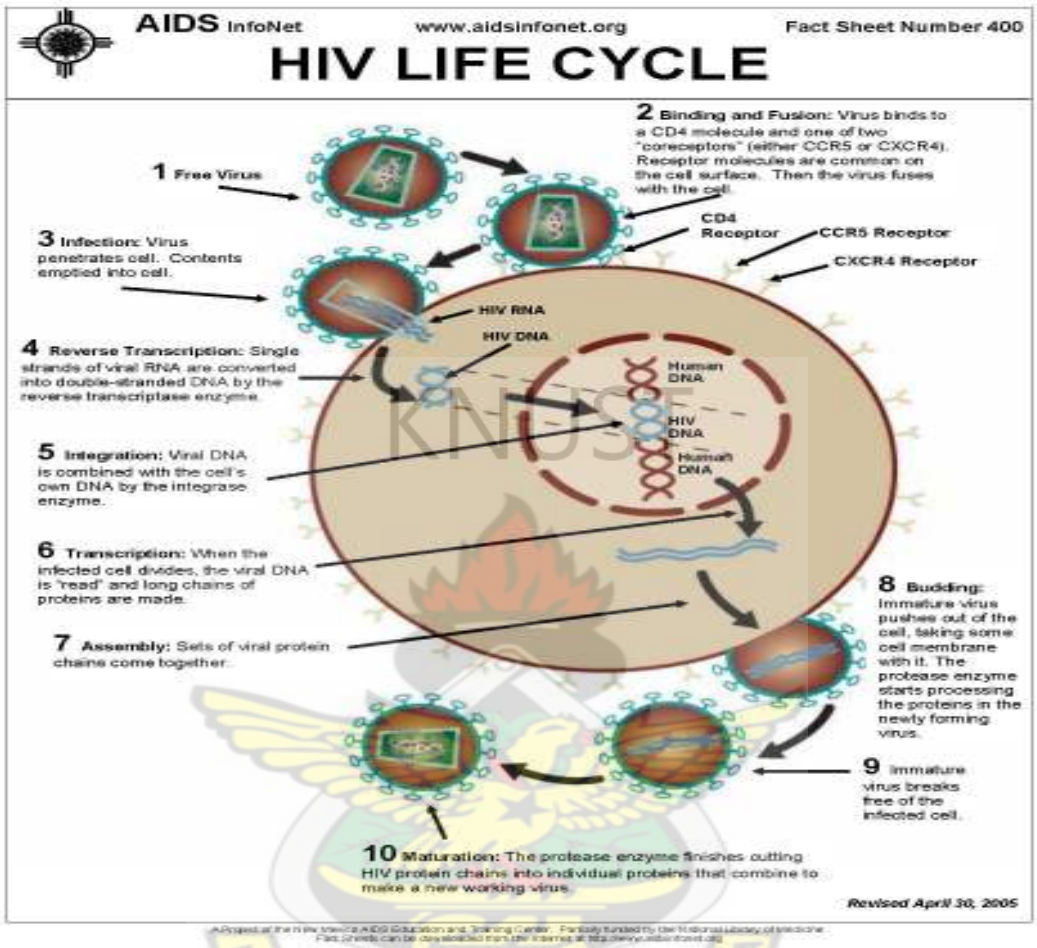





**2.6 Life Cycle of HIV**

A replication cycle of HIV begins with the virus identifying and binding to a susceptible target cell via a specific interaction between the viral gp120 envelope glycoprotein and the CD4 cell surface receptor CCR5 or CXCR4 (Klatzmann, 1984). Following binding, the HIV RNA penetrates and releases itself into the cytoplasm of the host cell where reverse transcription occurs with the help of the enzyme reverse transcriptase which converts the single stranded RNA to a double stranded DNA which become integrated into the host chromosomal DNA by the enzyme integrase to form a provirus. There is then the synthesis of viral structural proteins which then assemble to form virion that bud off the ruptured cell surface to continue the cycle as illustrated in the diagram below.

Figure 4. Schematic diagram of HIV replication




## 2.7 Geographical Distribution of HIV-1 Subtypes

HIV-1 subtype B is predominantly found in America, Japan, Australia, the Caribbean and in Europe (leitner, 1996) while subtype A and D are mostly in Sub-Saharan Africa (Kostrikis et al, 1995). Subtype C in South Africa, E in Central Africa Republic, Thailand and Southeast Asian Countries (Chakrabarti et al, 2000). Subtype F in Brazil and Romania while G and H occur mostly in Russia and Central Africa (Requejo, 2006)

## 2.8 HIV Transmission

Heterosexual intercourse is the most common mode of transmission. Other means of transmission include blood transfusion, mother-child transmission and the sharing if needles among others. A number of factors contribute to heterosexual transmission. Among these are;

- Frequent change of sexual partners
- Social vulnerability of women and young people.
- Presence of STI's and lack of proper treatment.
- Economic and political instability.
- Lack of knowledge of serostatus.
- Unprotected sexual intercourse.

### 2.8.1 Factors Affecting HIV Transmission

A number of factors are known to influence the transmission of HIV in humans. These are categorized into biological, cultural and socio economic factors.

Biological factors may include the infectiousness of the host which is influenced by the innate immune system. Naturally people with strong immunity tend to resist infections to a larger extent. The susceptibility of the recipient to the virus is also important to consider. The level of virulence of the type of HIV involved is also worth noting. HIV type-1 is more virulent and breaks down the individual faster than HIV type-2 which is less pathogenic (Kanki JP, 1999).

Socio economic factors that affect HIV transmission include mobility in the area of commerce or the global economy leading to widespread of the virus. Stigmatization has always been a problem in the fight against the HIV menace. Stigma prevents the acknowledgement of the problem and as such care seeking is prevented (Gregory et

al, 2002). Conflicts and the struggle for power also lead to displacement of individuals from their natural habitats and as such enhance the spread of the virus.

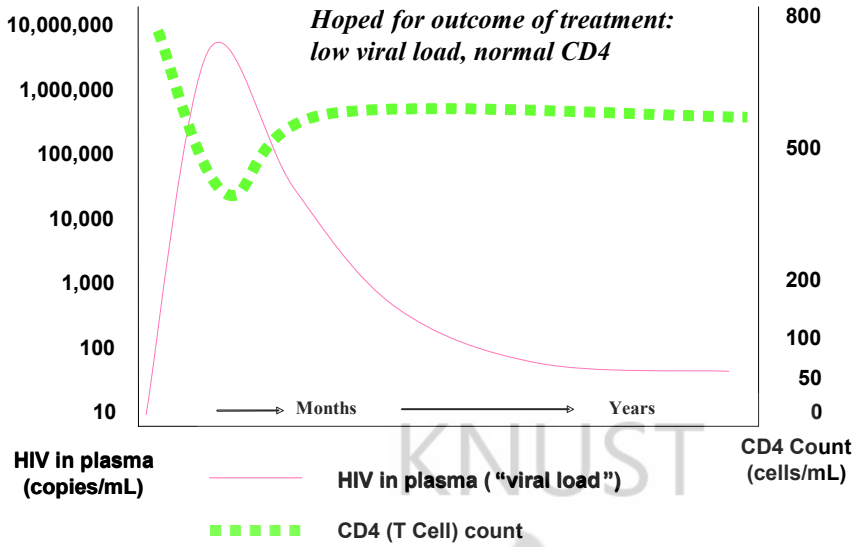
In a typical African setting, traditional beliefs prevent the acceptance of conventional medicine. Traditionalists attribute HIV/AIDS to witchcraft and as such patients would require the intervention of the fetish instead of the hospital. Other factors include gender discrimination, poverty and alcoholism (Gentilini and Chieze, 1990). Tradition sees nothing wrong with men with many sexual partners (Mane and Aggleton, 2002). As such, one man can infect many females unknowingly. The lack of information and illiteracy are also factors that fuel the spread of HIV.



**2.9 Course of HIV Infection**

The chronology of HIV-induced disease is associated with seroconversion and a period of intense viral replication with an abrupt decline in CD4 + T cell count in peripheral blood (Daar et al, 1991; Tidal and Cooper, 1991). The decrease in CD4+ T cell in circulation is probably due to the killing of HIV mediated cells or to the re-trafficking of cells to the lymphoid tissues and other organs (Fauci, 1993a). Patients then enter into a state of asymptomatic phase lasting for months to years. The extent of disease progression among HIV infected patients vary from one patient to the other (Pantaleo et al, 1995a; Cao et al 1995). Among the various factors that influence disease progression are ages, the genetic make up of the individual, virulence of the strain of virus as well as influence of other co-infected microbes (Evans, 1982). As the disease progresses, there is increasing amount of infectious viruses, viral antigens and HIV specific nucleic acids which correlates with the clinical course of the disease.

Figure 5. HIV progression and the immune system of an individual on HAART.




Source: HRSA HIV/AIDS Bureau, 2004

The Graph above is a representation of a typical course of HIV infection in relation to CD4+ T cell recovery in patients on HAART for a period of 10 years. During the period following primary infection, HIV disseminates widely in the body with an abrupt decrease in CD4+ T cells in the peripheral circulation. An immune response to HIV ensues, with a decrease in detectable viremia. A period of clinical latency follows, during which CD4+ T cell count continue to decrease, until they fall to a critical level below which there is a substantial risk of opportunistic infections.

**2.10 Opportunistic Infections**

The HIV virus type-1 and 2 cause serious disorder of the immune system by breaking down the normal immunity leaving the individual vulnerable to opportunistic infections and a variety of life-threatening infections including unusual malignancies.

Opportunistic infections usually set in with the invasion of pathogens, be it bacterial, virus, fungal or protozoa that will not under normal circumstance cause a disease in a normal person with a well functioning immune system. People with HIV/AIDS are susceptible to opportunistic infections. A number of factors influence the susceptibility. The main one is the suppression of the immune system, psychological stress and poor feeding habits.

Among the major opportunistic infections of HIV are Cytomegalo Virus (CMV) infection and the Mycobacterium tuberculoses (TB) infection which is recognized as the most important opportunistic infection in HIV (Frimpong et al, 1997). Where as in 1989, roughly 14% of Ghana's TB was attributed to AIDS, it is estimated that by the year 2009, about 59% of the projected TB cases will be due to HIV/AIDS epidemic (WHO Report, 2006). The occurrence of TB/HIV co infection results in increase in morbidity and mortality and it is a great public health concern.

Patients with advanced immunodeficiency remain at high risk of developing TB despite ART (Lawn et al, 2005a, b, Bonnet et al, 2006) as the function of immune system is not fully restored by ART (Sutherland et al, 2006).

Co-infections with other pathogens also increase the HIV viral burden thereby accelerating the disease progression in the patient. It is against this background that in poor countries including Ghana where access to ART is not of a generalized use, it is recommended that high quality feeding be offered as an alternative to enhance the lives of the people.

## 2.11 Laboratory Diagnosis of HIV Infection

The HIV virion contains two identical copies of RNA and a number of viral proteins. Antibody response to these viral proteins including products of viral genes env (gp160, gp120 and gp41), gag (p41, p17 and p9) and pol (p32, p66, p51and p11) can be detected in serum, plasma and whole blood. Serological testing for HIV antibodies remains the most effective approach to HIV screening with Enzyme immunoassays (EIA) being the most commonly used method (Yilmaz, 2001).

In a classical EIA, immobilized HIV antigens are used to bind anti-HIV antibodies from the patient's blood sample. Bound anti-HIV antibodies are then complexed with enzyme-labelled anti-human IgG and are detected in a colourimetric reaction. The resulting colour change is quantified spectrophotometrically and is proportional to the concentration of antibodies in the original sample (Gurtler 1996, Bylund et al, 1992).

There has been a marked improvement in the performance of EIA since 1985 with the introduction of 3$^{rd}$ generation double antigen assays that use recombinant proteins and synthetic peptides which are more specific than the formerly used 1$^{st}$ which used whole viral lysate as a source of HIV antigen. The time interval between infection and antibody detection has been shortened in the process (Schupback, 2003).

Presently, 4$^{th}$ generation EIA's are available and they detect HIV antibodies and p24 antigens in a single assay. Thus shortening the window period to an average of 7 days (Brust et al, 2004).

## 2.12 Rapid/Quick Tests

A variety of rapid EIA screening tests are also available for qualitative detection of HIV antibodies in blood. These are based on one of four immunodiagnostic

principles; particle agglutination, immunodot (dipstick), immunofiltration and immunochromatography (Branson, 2003; Giles et al 1999).

These are mostly one-step tests with inbuilt controls and a shorter turn around time. They are easier to be performed and require little in terms of equipment (WHO, 2004). Rapid tests are appropriate for testing single or few samples where as the EIA's are suitable for analyzing large numbers of samples and are suitable for blood screening centres since they are highly cost effective (Yilmaz, 2001).



**2.13 Confirmation Tests**

A repeatedly reactive screening results demands confirmation by Western Blot or a Line Immuno Assay according to World Health Organization standard algorithm of HIV testing (Tamashiro, 1993). These detect the various antigens of the HIV virus and differentiate between the various HIV types. Alternatively, nucleic acid-based testing like Polymerase Chain Reaction (PCR) is more definitive as it detects directly the DNA of the virus. However, it is more expensive and demand sophisticated machinery, hence, it can not be of a generalized use in resource-constraint areas.

**2.14 CD4 (Cluster of Differentiation) Cell Count**

The CD4 cell is a T lymphocyte cell and constitutes the backbone of the cell-mediated immune system. T cells respond to viral antigens via surface receptors (CD4 cell) leading to a series of cellular morphological and biochemical changes. The measure of the CD4 cell count at a point in time is a desirable marker to monitor the level of breakdown of a functional immune system and directly reflect the state of disease progression in an individual. The natural history of HIV disease progression in an individual is characterized by a progressive loss of CD4 T lymphocytes As the

CD4 cells decline, the immune system is weakened and the risk of contracting opportunistic infections increases (Vergis and Mellors, 2000).

### 2.15 Measure of CD4 Cell Count

The CD4 count is either measured in absolute numbers or by percentage cell count values. The absolute number gives the specific level of immuno suppression with age, usually used in adults. The percentage count places an individual in an immunologic category, which is a better marker of identifying disease progression in young children.



HIV RNA levels in perinatally infected infants are generally low at birth (<10,000 copies/ml); increases to high values at age 2 months and decreases slowly after the first 2 years. In line with this, CD4 values in healthy infants who are infected are considerably higher than those observed in even uninfected adults and slowly decline to adult values by age 6 years. Other minor infections also affect the CD4 count of an individual hence leading to variations in relation to time. It is therefore advisable to measure it when the patient is clinically stable.

#### 2.15.1 CD4 Kinetics

The CD4 cell is the primary receptor of the gp120 of HIV (Dalgleish 1984, Klatzmann, 1984). It is a monomeric glycoprotein that can be detected on the cell surface of about 60% of T-lymphocytes, on T-cell precursors within the bone marrow and thymus, and on the monocytes and dendritic cells and microglial cells of the central nervous system. The point of binding overlaps the part of the CD4 where its natural ligands – HLA class II molecules are located. HIV attaches to the T cell receptor complex (TCR) on CD4+ T-cells and bind to HLA class II molecules on

antigen presenting cells. The binding of gp120 to CD4 is not only a crucial step for viral entry but also interferes with intracellular signal transduction pathways and promotes apoptosis in CD4+ T-cells (Banda, 1992). However, from the experiments performed by Edwards et al in 2001, using isolates of HIV-1, HIV-2 as well as SIV, human cells were successfully infected independent of CD4. This observation thus suggests that CD4 is not a prerequisite for entry by these viruses.

### 2.15.2 Mechanism of Functional and Quantitative Depletion Of CD4 + T Cells

HIV infects and kills CD4+ T lymphocyte cells (Clark et al, 1991). A number of proposed mechanisms involved in the destruction of the lymphocyte cells could either be direct or indirect. Direct mechanisms include the disruption of the cell membrane as HIV buds off from the surface in an HIV-mediated cytopathic effect in a single cell killing, HIV-mediated formation of giant cells called syncytia, antibody dependent cellular cytotoxicity and by apoptosis (Pantaleo et al, 1993a) resulting in the killing of CD4+ T lymphocyte cells.

In the case of indirect mechanism, uninfected cells fuse with infected cells leading to the death of affected CD4+ T cells ( Fauci, 1993a). The fusion result in the formation of syncytia associated with cytopathic effect of the HIV (Sodroski et al, 1986; Lifson et al, 1986).

Uninfected cells may be killed when free sgp 120 binds to their surfaces marking them for destruction by antibody-dependent cellular cytotoxicity response (Lyerly et al, 1987). According to the findings of the investigations by Janeway in 1991 and Hugin et al, 1991, HIV super antigens may also stimulate and trigger massive expansion of CD4+ T cells, killing them in the process. The ultimate induction of a form of programmed cell death called apoptosis has been proposed as an additional

mechanism for CD4+ T cell loss in HIV infections (Ameisen and Capron, 1991; Terai et al, 1991). Recent reports indicate that apoptosis occurs mostly in peripheral blood mononuclear cells (PBMCs) and in lymph nodes in a greater number of HIV infected individuals than in non-infected persons (Finkel et al, 1995; Muro-Cacho et al 1995). Precursors of CD4+ T cells in the bone marrow and thymus are destroyed by the HIV virus when the micro environmental conditions of these organs necessary for optimal sustenance and maturation of the progenitor cells are affected (Schnittam et al, 1990b; Stanley et al, 1992). Moreover, it has become evident from research that there is a substantial viral burden and active viral replication in both peripheral blood and lymphoid tissues, even in early stages of HIV infections (Fox et al, 1989; Coombs et al, 1989; Embretson et al, 1993). It has been reported that 25% of CD4+ T cells in lymph nodes of HIV infected individual harbor HIV DNA early in the course of the disease (Embretson et al, 1993). Further research works by Wei et al and Ho et al both in 1995 also suggest that in HIV infections, there is a continuous production of new viruses such that there is destruction and replacement of 1 billion CD4+ cells per day.



**2.16 Clinical Application of CD4 Count**

Absolute CD4 + T lymphocyte cell count is used to evaluate the immune status of patients with suspected immune deficiencies. The likelihood of a disease progressing to AIDS or death without the intervention of antiretroviral therapy increases with increasing immunodeficiency (Mellors et al, 1997).The CD4 antigen is the receptor for HIV. The cell count at a point in time is the desired cellular parameter most closely associated with HIV disease progression and patient prognosis.

## 2.17 Chemokine Receptors

Chemokine receptors CCR5 and CXCR4 have also been found to be co receptors for HIV entry in infected individuals and that their presence suppresses the replication of some viral isolates tested (Cocchi 1995). Apart from CCR5 and CXCR4, other chemokine receptors like the CCR3, CCR2, CCR8, CCR9, APJ and ChemR23 have been identified and shown to enhance the entry of certain HIV isolates (Deng 1997, Liao 1997). Despite this broad spectrum of potentially available co receptors, CCR5 and CXC4 seem to represent the most relevant co receptors for HIV-1 in vivo.



## 2.18 Viral Load

Viral load indicates the prognosis of HIV infection and provides evidence of the virological response to therapy as it measures the amount of measurable virion in circulation. Studies have demonstrated that following the administration of effective ART, there is a drop of HIV-1 RNA (Ho et al, 1995).

There are two main types of viral load testing; molecular techniques and viral culture. Molecular techniques such as PCR assays measure HIV genome called RNA from circulating blood. If a viral load assay result is undetectable, it may imply that the reading is below the level of detection but not necessarily that there is no virus in the system. The total body viral load can not be directly gauged since there are over 80% culturable virus hidden in the bone marrow, lymphoid tissues and organs which are hidden and can only be measured by viral culture (Wei et al, 1995). Viral loads are used to provide definitive indication of response to antiretroviral therapy. The efficacy of ART is strongly associated with changes in the quantity of plasma level of HIV-1 RNA (Katzeenstein et al, 1996). They are therefore usually requested when there is treatment failure to a particular drug combination. Even though this test is

important, it is not critical for ART monitoring especially if there is clinical improvement, good adherence and increase in CD4 count. Moreover, the test can not be done routinely as a result of financial constraint. It is recommended that where available and affordable it should be done at month 0, 3, 6 month then six-monthly. If the viral load becomes undetectable and there is good adherence to treatment, the frequency of its determination can be reduced unless there are indicators of deterioration (NACP Report, 2005).



**2.19 HIV in Neonates**

Not all infants born to HIV-positive mothers become infected with the disease, only about 15-45% of them become infected either before or after birth (Lambert et al., 1990; Lindgren et al. 1991; Hutto et al., 1991). However, infants born to HIV-positive mothers carry detectable antibodies which may persist for 15 months. Hence, the presence of antibodies in neonates may not represent a true HIV infection (Newell et al, 1995). In the few infants who acquire HIV and develop AIDS, the rate of disease progression vary directly with the severity of the disease in the mother at the time of delivery (European Collaborative Study, 1992; Blanche et al, 1994). DNA testing using PCR is the last resort which of course is not common to be undertaken in resource-constraint countries including Ghana due to its complexity, non availability and the high cost involved.

**2.20 Staging of HIV/AIDS**

Staging is usually done either by using the clinical presentation or laboratory markers. A combination of the two gives a fair idea to categorize the immunosuppression of the individual patient which in turn directly reflects the drug combination appropriate for the patient. This staging system is proven to be reliable in predicting morbidity or

mortality of the infected individual. This is because they are based on clinical markers believed to have prognostic significance essential to monitor patient's treatment performance (WHO, 1990). The clinical staging is useful for the assessment of baseline or entry into long-term HIV care of patients in treatment programmes. It provides guidance as to decision making in the start of co-trimoxazole prophylaxis and other HIV related interventions, including when to start ART. The clinical staging is related to survival, prognosis and progression of clinical disease without ART intervention in patients (Badi et al, 2001. Dilys et al, 2002. Fahey et al, 1990)



Clinically, about four categories are identified;

**Stage 1**; Asymptomatic, No related symptoms, generalized lymphadenopathy

**Stage 2**; Mild Symptomatic, Weight loss < 10% body weight, oral ulcerations, Fungal infections, herpes zoster,

**Stage 3**; Advanced symptoms, Weight loss > 10%, chronic diarrhoea or fever, pulmonary TB, Severe bacterial infections, persistent oral candidiasis, recurrent upper respiratory tract infections.

**Stage 4**; Severe Symptoms, AIDS defining illness; HIV wasting syndrome, Pneumocystis pneumonia, brain Toxoplasmosis, candidiasis, oesophagitis, extra pulmonary TB, CMV, Retinitis, Kaposi sarcoma, bed ridden > 50% of the day during the last month.

On the other hand, the CD4 axis is based on a range of values. Generally three groups are recognized;

**Group A**; CD4 > 500 cells/ul (CD4%>28%)

**Group B**; 200 <CD4 < 500cells/ul (14 %< CD4 %< 28%)

**Group C**; CD4 < 200cells/ul (CD4 %< 14%

**Table 1. WHO Staging of HIV/AIDS**

| Laboratory Axis | | | | Clinical Axis | | |
|---|---|---|---|---|---|---|
| **Lymphocytes** | | CD4 | Stage1 Symptomatic | Stage 2 Early HIV | Stage 3 Intermediate | Stage 4 Late AIDS |
| **A** | **>2000** | **>500** | 1A | 2A | 3A | **4A** |
| **B** | **1000-2000** | **200-500** | 1B | 2B | 3B | **4B** |
| **C** | **<1000** | **<200** | **1C** | **2C** | **3C** | **4C** |



Blue colored areas refers to progression to AIDS

Reference Ranges - Total Lymphocytes; 1500-4000/$mm^3$

CD4 Count; 450-1400cells/ul

**2.21 National Policy for Treatment Criteria**

Adults;

WHO clinical stage III or IV

CD4< 250

Children;

Symptomatic children in pediatric stage I and III whose mothers are HIV positive.

CD4 < 20% in child less than 18 months

CD4 < 15% in child more than 18 months

## 2.22 Mode of Action of Antiretroviral Drugs

ARVs act on HIV by interfering with its reproductive cycle. The main points of action are;

1. To inhibit the enzyme reverse transcriptase production thereby interrupting the synthesis of provirus DNA. Thus ARVs prevents the formation of DNA. NRTI and NNRTI act here.

2. To inhibit maturation of the virion by interrupting the protein processing and virus assembly. During this stage, protease enzymes are required and protease inhibitors act here.

3. Fusion inhibitors which prevent the recognition, attachment and penetration of the virus to the target cell.

## 2.23 Goals of Antiretroviral Therapy

Currently there are 16 approved ART agents for HIV-1 infection (in the USA) from which WHO recommends that resource-constraint countries select their first line regimen from.

The Aim of Antiretroviral Therapy is to;

(1) Virological goal; Reduce the detectable viral load of HIV RNA as low as possible, below the current level of detection of 50 copies/ml.

(2) Clinical goal; maintain this level of suppression for as long as possible to reduce mortality, morbidity and improve quality of life.

(3) Immunological goal; Restore and preserve immunological function and delay clinical progression of HIV disease, thus preventing opportunistic infections from occurring.

(4) Epidemiological goal; Prevent HIV transmission - PMTCT

Several years ago it was thought that HIV eradication was theoretically possible if HAART (Highly Active Antiretroviral Therapy) was able to control viral replication for a period of 2 - 3 years. More recent data shows that there is still ongoing low level HIV replication even when plasma HIV RNA is below the current level of detection (i.e. below 50 copies/ml) which results in some "reseeding" of lymphocytes. Further, data on the apparent half life decay of resting memory CD4 lymphocytes infected with latent HIV pro-virus have been revised, and are now considered to be at least 6 months, and may even be as long as 44 months. This means that HIV eradication using current antiviral therapies will be expected to take at least a decade or longer, if such a goal is indeed possible at all.

The realistic view then is that antiretroviral therapy should be considered long-term management of a chronic viral infection. Current available drugs approved for treatment are in three mechanistic classes. Treatment has evolved from single therapy through dual nucleoside analogue therapy to the current tri therapy with two nucleoside analogues in combination with a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor.

**2.24 Elements of Antiretroviral Therapy (ART)**

Essentially, ART has dramatically increased the survival of HIV-infected patients (Palella et al, 1998) and prevented vertical transmission of HIV (Connor et al, 1994). Basically, the elements of an effective ART program involve access to Voluntary counseling and testing (VCT). This could either be within a health facility, as a stand alone or integrated into a care provision.

The availability of trained personnel in terms of knowledge, skill and attitude is very important in this respect to diagnose and treat common HIV-related illnesses and manage ART, observing the principle of chronic disease management and in accordance with national or international guidelines. By this, clinicians, nurses, pharmacists, laboratory professionals and nutritionist among others, must be adequately trained for the program.

Access to laboratory services with the capacity to do routine tests like complete blood count, liver function tests, CD4 count (a desirable test) or alternatively, total lymphocyte count. In some situations, it is important to measure viral load (optional) if possible, these are very essential for an effective ART program. The laboratory should therefore be well equipped with the necessary facilities to be able to provide timely, accurate and reliable results. Above all, there should be a consistent supply of antiretroviral drugs, as well as drugs to prevent and manage opportunistic infections and other HIV-related illnesses, and drugs for palliative care. There should be a mechanism for the regular order, storage and distribution of these drugs to ensure sustainability.



**2.25 National Policy for Antiretroviral Drug Regimen**

The regimen is a triple therapy. The following is recommended;

- 2 Nucleoside Reverse Transcriptase Inhibitors (NRTI) and1Non-Nucloside Reverse Transcriptase Inhibitor (NNRTI).
- 2 NRTIs and 1 Protease Inhibitor (PI)

- 2NRTIs and 2PIs. The 2 PIs are considered as one ARV, as the second PI, usually ritonavir, is in low dose and is used to boost the blood level of the first PI.

The first line regimen is the first option for treatment of all patients who fit the treatment criteria. The second line regimen is used when there is clinical evidence of treatment failure with the first which is confirmed with either CD4 count monitoring or possibly viral load if available.



**2.26 Initiation of ART**

**First Line;**

Zidovudine 300mg + Lamivudine 150mg; one tablet two times a day.

Neverapine 200mg; one tablet daily for two weeks. If there are no adverse reactions at this stage, the dosage is increased to one tablet two times a day.

**Second Line;**

For patients who develop severe adverse side effects to Zidovudine, Stavudine will be used in its place. These patients will therefore receive Stavudine, Lamivudine and Nevirapine.

For patients who react to Nevirapine or experience severe drug reactions, Efaverenz will be used instead. (NACP/MOH/GHS, 2005)

**Inclusion Criteria**

Antiretroviral therapy may be initiated when the patient satisfies the following criteria;

1. Patient with CD4 count less than 250 cells/ml ( Ghana National Policy) (WHO, 2004) and or

2. Symptomatic with HIV infection in WHO stage 3 and 4.

**Exclusion Criteria**

ART should not be initiated under the following circumstances;

1. If the patient is not motivated or does not show real interest or commitment in starting the treatment.
2. Patient does not complete pre-treatment adherence counseling.
3. Treatment is not sustainable
4. Patient has any acute opportunistic infection which must be treated before initiation of therapy.
5. No laboratory monitoring services (NACP, 2005)



**2.27 Adherence**

Adherence to HIV antiretroviral therapy is probably the most important variable that predicts the ability to achieve and maintain suppression of viremia to below the detectable level.

The term adherence refers to the ability of a person living with HIV/AIDS to be involved in choosing, starting, managing and maintaining a given therapeutic combination to control HIV viral replication and improve immune function. This is achieved through a mutual agreement on a treatment plan between the clinician and the patient working together on a concept acceptable to both parties. Non adherence to therapy often leads to treatment failure (Turner, 2002). Insufficient plasma levels of ARV's and partial suppression of viral load are conditions under which resistance

can develop. A higher level of compliance is associated with lower risk of resistance (Sethi, 2003).

Most often, patients who do not adhere to therapy are those with substance or alcohol abuse or with side effects. Studies have shown that patients with depression and those without any social support are at high risk of non-adherence (Murri, 2001; Frank, 2002). Poor adherence leads to virologic failure and immunologic decline (Mannheimer, 2002) and a higher risk of mortality (Garcia, 2002).



**2.28 Treatment Failure**

Failure to treatment could be defined in terms of clinical, immunologic or virologic. Clinical failure looks at persistent disease progression with the development of opportunistic infections or malignancy when the drugs have been given enough time to induce a protective degree of immune restoration.

Immunologic failure considers the fall in CD4+ cell count higher than 30% from the peak value or a return to, or below, the pre-therapy baseline while the virologic failure refers to the failure to achieve undetectable viral load levels after 6-12 weeks; a repeatedly continued detection of viremia in an individual is an indicative of incomplete viral suppression.

**2.29 Side Effects of Antiretroviral Drugs**

Side effects also known as toxicities or adverse reactions are the effect of the drug that is not intended. In addition to drug resistance and the difficulty of adhering to complex regimens, side effects associated with highly active antiretroviral therapy (HAART) have become a major concern.

Side effects due to anti-HIV drugs are not new. Since the late 1980s, the earliest drugs used to treat HIV infection--AZT (Retrovir) and other nucleoside analogs,

generally at higher doses than are currently used were associated with several adverse events including nausea, diarrhea, muscle disease (myopathy), and hematologic effects eg. bone marrow suppression leading to anemia and low white blood cell counts ( De Jesus et al, 2004)

However, the most recently approved class of anti-HIV drugs; the protease inhibitors have been associated with a new set of strange side effects including metabolic abnormalities and changes in body fat distribution (lipodystrophy), a condition associated with both clinical and metabolic alterations (Behrens, 2000). Patients experience loss of subcutaneous fat (lipoatrophy) in the face (periorbital) limbs and buttucks. Research has shown that there is an initial increase in limb fat in the first few months after initiation of ART followed by a progressive decline over ensuing years (Mallon et al, 2003). The magnitude and unusual nature of HAART-associated adverse effects have contributed to the current rethinking and debate about anti-HIV therapy. Indeed, these side effects are worrisome enough that some physicians are questioning the value of early treatment, and researchers have begun to explore new treatment strategies that allow for the use of fewer drugs for shorter periods.

The occurrence of side effects can vary dramatically among different people. Some people experience frequent and severe adverse reactions that necessitate dose reductions or discontinuation of treatment (Carr et al, 2001), others have side effects that are uncomfortable or annoying and can interfere with their daily quality of life; still others experience few or no adverse reactions. Although it is impossible to predict in advance who will experience side effects, adverse reactions do tend to be especially severe in people with advanced HIV disease.

It is important to note that many types of symptoms associated with the use of anti-HIV drugs including peripheral neuropathy, gastrointestinal symptoms, mental symptoms, and certain metabolic changes are also found in people with HIV infection who are not taking anti-HIV therapy, especially those with later-stage disease and/or high viral loads. Sometimes it is difficult to determine whether a symptom is related to HIV infection or whether it is a drug side effect. Below is a list of some ART's and their side effects;



| **Drug** | **Adverse Effect** |
|---|---|
| Zidovudine (AZT) – | anemia |
| Lamivudine (3TC) – | hypersensitivity reactions |
| Combivir (3TC + AZT) – | Stevens Johnson Syndrome |
| Stavudine (d4T) – | peripheral neuropathy |
| Kaletra – | diarrhoea |
| Nelfinavir -- | diarrhoea |

In addition to this, some people are unable to tolerate certain drugs and develop allergic or hypersensitivity reactions; such reactions seem to be more common in people with AIDS than among the population as a whole. Hypersensitivity reactions can range from skin rashes and swelling to life-threatening anaphylactic shock characterized by difficulty breathing and a rapid decrease in blood pressure. This has been reported in people who take abacavir, the most recently approved nucleoside analog, and these patients experience a severe hypersensitivity reaction characterized by nausea, fever, fatigue, and flu-like symptoms, which may be followed by a

generalized measles-like rash. Other drugs noted in this respect are nevirapine, ritonavir, and sulfa drugs.

**2.30 Counseling**

Counseling is very essential for active ART dispensation. The objective here is to help clients to recognize and understand their own problems, to be able to express their thoughts, feelings and emotions so that they can be guided to make wisely, an informed decision and to help change their inappropriate behaviors. A counselor should fulfill some basic qualities; He should have a total command over the pros and cons of HIV/AIDS, be trust worthy, friendly, respectful and confidential. Counseling is done at various stages of the ART process; Pretest counseling which is aimed at adequately preparing client for HIV test. This ensures that client's anxiety is reduced markedly so that one can make a choice as to whether or not to undertake the test.

Post test counseling is to help clients understand and accept the results of the test done so that one can adjust to the conditions of the infection and plan towards the future. Post test counseling also gives psychosocial support to the client.

Counseling for ARV therapy looks at the advantages and disadvantages of the ARV drugs and emphasizes on the lifelong use of the drugs, adherence, compliance, mutual agreement, drug resistance, side effects, obstacles and the readiness of the client to start the therapy, information on drug administration, mechanism of action and the impact on the client's family (Stenson et al, 2005).

# CHAPTER THREE

## 3.0 Materials and Methods

### 3.1 Study Setting

The study was carried out at the Serology Unit in collaboration with the HIV clinic of KATH in Kumasi after obtaining ethical clearance from the committee of Human Research and Ethics of the Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching hospital.

### 3.2 Study Subjects

Subjects for this study were all adult HIV infected patients enrolled to be on anti retroviral therapy (ART) at the KATH HIV Clinic between the period December 2003 and August 2004 and who gave their consent to participate in the study. It was limited to this period because it was only at this period that patients with HIV infection could be serotyped as HIV-1, HIV-2 or HIV-1 & 2 dual infections. Criteria for admitting patients into the study included being 18 years and above, known to be treatment naïve, have CD4 cell count less then 250 cells/ul and or in WHO stage III or IV of HIV infection. In all, a total of 108 participants who qualified for HAART were recruited for the study.

### 3.3 Data Collection

Patient's data was collected into a specifically designed form (appendix 2). Information collected included demographic data such as age, sex, marital status, educational level, religion, economic status and support. Their HIV results and type were also included and where positive and put on therapy, their CD4 cell count reading before initiation of therapy and while on therapy also recorded. At the end of the study, these data were fed into a computer software programme Epiinfo 2003

version 3.3 (CDC, Atlanta) and analyzed using Stata Intercooled version 8 (Stata Corp, USA) and Excel Windows XP 2007 (Microsoft Corporation, USA).

### 3.4 Method

Five (5) milliliter venous blood was taken from each patient who reported to the Serology laboratory for HIV test during the period under study into a sterile tube with a screw cap for HIV screening using Vironostika HIV Uni-Form II plus O (Biomerieux, Holland). All samples that tested positive with Vironostika were confirmed with Rapi Test (Morwell Diagnostics GmbH, Switzerland) as a supplementary test that further serotyped all positive cases into HIV-1, HIV-2 and HIV-1 & 2. All patients who were declared seropositive and who qualified for HAART were tested for their pre-treatment CD4+ T cell count by using the FACSCount (Becton Dickinson, USA) and their values recorded. The patients were then followed up with consecutive CD4 count determinations every six months after initiation of HAART and their values recorded over a period of two years.

### 3.5 Specimen Collection

Serum or plasma was used. No special preparation or fasting prior to the taking of the specimen was needed. The blood specimens were taken by venipuncture into 4.5ml vacutainer tubes in accordance with standard laboratory procedures. Fresh specimen were stored at -2° to -8°C for up to a week and the serum aliquots stored at -20° C. Specimen were not subjected to more than one cycle of freeze/thaw to avoid contamination.

### 3.6 HIV Testing

#### 3.6.1 Test Procedure for Vironostika

A template sheet was prepared with all the labels of the test samples and the controls. The required number of test strips were removed from the sealer and fit into the micro plate holder. Hundred (100)ul of specimen diluents was dispensed into each well including the control wells. Fifty (50)ul of sample and controls were dispensed into corresponding wells and agitated by using the micro plate shaker to ensure thorough mixing. The plate was incubated at 37◦ C for a period of 1 hour. At the end of the incubation, the plate was washed for six cycles with phosphate buffer avoiding overflow and cross contamination and blotted on an absorbent tissue to dry. Hundred (100)ul of the TMB substrate was dispensed into each well agitated for even mixing and incubated at 37◦C for the next 30mins in the dark. The reaction was then stopped with 100ul sulfuric acid ensuring that the same pipetting sequence and time interval was maintained. The plate (Figure 6) was tapped to ensure thorough mixing and read at 450 nm wavelengths using a spectrophotometer within 15 minutes. The relative absorbance or optical densities (OD) for each specimen were recorded and the cut-off (CO) value computed.


Figure 6. Microplate ELISA for HIV antibody: colored wells indicate reactivity

### 3.6.2 Interpretation of Results

Qualitatively, all wells that developed strong yellow colouration as indicated in figure 6 above were said to be reactive and that those samples tested either contained antibodies to H IV-1 and or HIV-2 or HIV-1 group O. On the other hand, the non coloured wells were non reactive and the sample tested were either negative controls or did not contain anti-HIV-1, anti-HIV-2 or anti-HIV-1 group O or that the sample contained one or more of these below the detection limits of the Vironostika assay. The relative absorbance is directly proportional to the colour intensity developed in the wells. The presence or absence of detectable antibodies or antigens to HIV-1 and or HIV-2 was quantitatively determined by comparing the optical density (OD) to the cutoff value (CO) calculated by adding 0.100 to the mean of the negative controls (NCx), i.e. 0.100 + NCx. Conventionally, a test sample is reactive if the sample OD is grater than the cutoff value and non reactive if the sample OD is less than the cutoff value.



### 3.6.3 Validation of Test

An assay run was valid if the first positive control (PC1) minus the mean of the negative control (NCx) was greater or equal to 0.400 (i.e. PC1 – NCx ≥ 0.400) or the second positive control (PC2) minus the mean of the negative controls was greater than 0.400 (i.e. PC2 – NCx ≥ 0.400)

### 3.7 Test Procedure for Rapi Test HIV-1 & HIV-2

10ul of serum or plasma or 20ul of whole blood was added to the sample well and allowed to sink. Three drops of assay diluents were added. Approximately 5 to 20 minutes was allowed for the reaction to complete. The appearance of a second band at mark '1' in the result window indicated that the sample was reactive with HIV-1 and all such cases were typed as patients having HIV-1 alone. On the other hand, all reactive cases that showed on mark '2' were typed as HIV positive cases with HIV-2 alone infection. In much the same way, all cases with bands on both '1' and '2' were typed as HIV-1 & 2 dually infected patients.



### 3.8 Facscount for CD4 Determination

The FACSCount System is a Becton Dickinson's automated instrument (Figure 7) and reagent kit designed to enumerate the absolute CD4+, CD8 and CD3 T lymphocytes in whole blood. It is a compact cell counter with a built-in computer. A sample holder lifts a sample tube to the sample ejection probe. There is a system fluid reservoir and a waste reservoir, equipped with a liquid level detector to indicate full and empty conditions. A laser beam intersects the sample stream within a flow cell.

Figure 7 Becton Dickinson FACSCount machine at KATH Serology Laboratory.

### 3.8.1 CD4 Count- Test Procedure

Whole blood samples were collected in 4.5ml BD K2E vacutainer tubes and mixed adequately with the EDTA by gently inverting up and down for at least ten times. The test vial was vortexed upside down and then upright for 5 seconds each to ensure even mixing. 50ul of whole blood was added to the vial using the back pipetting technique with an automatic pipette and vortexed again for 5 seconds before incubating in the dark for a period of 1 hour. At the end of the period, the product was fixed with a fixative and vortexed to ensure even mixing. The product was then fed onto the Facscount automated reader for analysis and the CD4/CD3 results was printed out automatically. Four consecutive CD4 counts were determined following a similar procedure every 6 months over a two year period and recorded.

### 3.8.2 Mean CD4 Count Computation

A mean pre therapy CD4 count was computed for HIV-1 infected patients, HIV-2 infected patients and HIV-1 & 2 dually infected patients using Microsoft Excel software programme. A similar procedure was followed in the computation of mean CD4 count for 1$^{st}$ post therapy, 2nd post therapy, 3$^{rd}$ post therapy and 4$^{th}$ post therapy mean CD4 values.

# CHAPTER FOUR

## 4.0 Results

In an attempt to determine how people infected with HIV-2 respond to treatment specifically designed for HIV-1, I looked at the CD4 T-cell kinetics of HIV-2 patients and compared with HIV-1 infected patients. I also looked at the patient specific factors that affect their response to therapy such as age, sex, marital status, educational levels, etc. In all, a total of 108 HIV positive but treatment-naive patients were enrolled into the study. They included 95 HIV-1infected patients, 7 HIV-2 infected patients and 6 HIV-1 & 2 infected patients.



## 4.1 Demographic Characteristics of Population Studied

### 4.1.1 Age Distribution among patients

Out of the 108 patients, 45(41.7%) were males while 63(58.3%) were females. Age distribution was over a wide range, spreading between a minimum of 19 and a maximum of 72 years. Forty-seven percent (40.7%) were aged between 40-49 years with less than 1% occurring in the 10-19 years group.

Table 2. Age Distribution of Studied Population

| AGE | 1-9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 |
|---|---|---|---|---|---|---|---|---|
| NUMBER | 0 | 1 | 11 | 36 | 44 | 8 | 6 | 2 |
| FREQUENCY (%) | 0 | 0.9 | 10.2 | 33.3 | 40.7 | 7.4 | 5.6 | 1.9 |

### 4.1.2 Marital Status of Patients

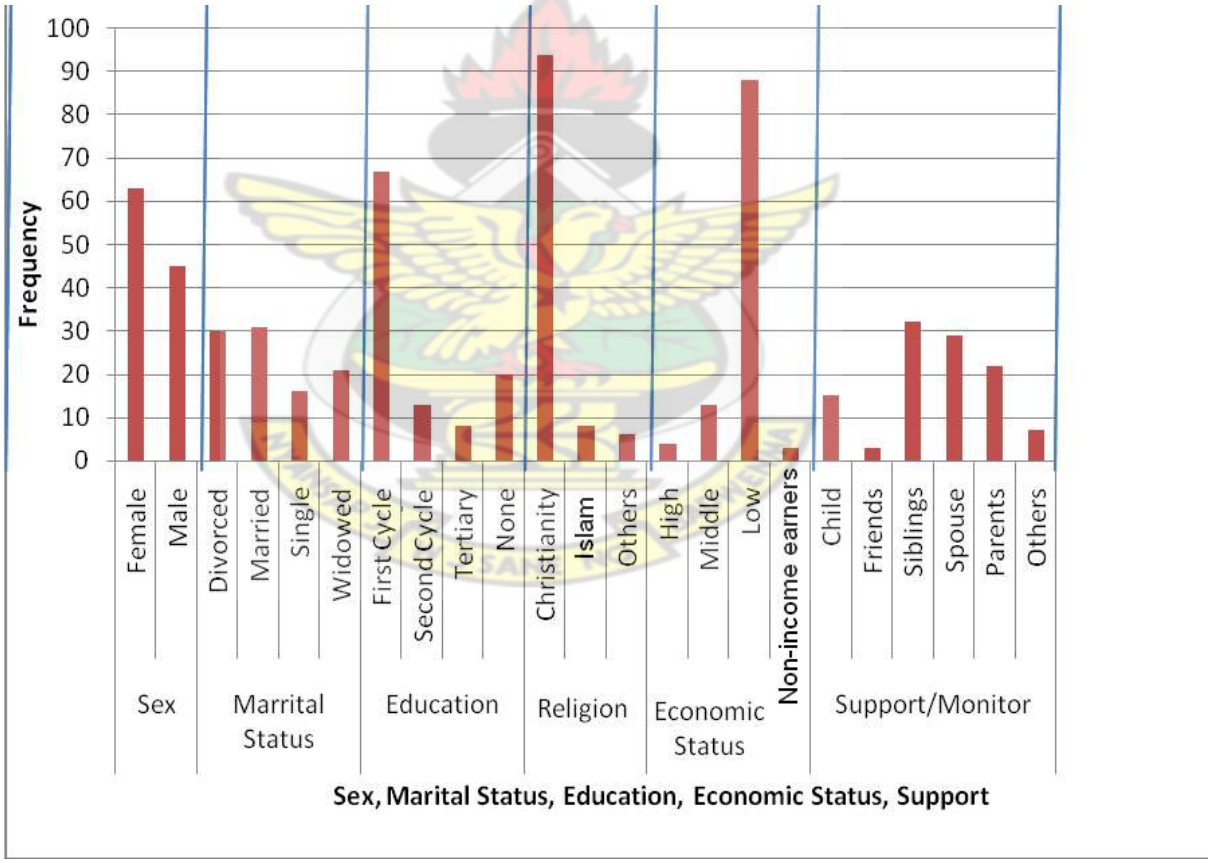
On marital status, 14.8% were single, 28.7% were married and 27.8% were divorced. 24.1% were widowed (Figure 8).

### 4.1.3 Economic Status of HIV patients

Professionally, the patients were of varied employment backgrounds including civil servants, traders, skilled labourers such as drivers, mechanics, masons, hairdressers, carpenters and . About 88 of them (81.5%) were low income earners, earning less than 2400 Ghana cedis per annum while 13(12%) were middle income earners and taking between 2,400 – 6,000 Ghana cedis with 4(3.7%) being high income earners, earning more than 6,000 Ghana cedis per annum. (Fig. 8)



**Figure 8. Graphical presentation of demographic data**



### 4.1.4 Educational Levels of Patients

Looking at the educational background of the patients, only 7.4% were within the tertiary educational level with just about 12.0% having completed second cycle institution with as many as 81.5% either completed first cycle education or never attended school before (Fig. 8).

### 4.1.5 Support/monitor of patients

To determine how the patients were accepted by society without being stigmatized, we looked at whether they had people supporting or helping them on therapy.

Here, results revealed that those taken care of by siblings were 29.6%, parents (20.4%), spouse (26.8%) children (13.9%) and others comprising of landlords, external family members, church members etc. being 6.5% (Fig. 8).

### 4.1.6 Religious Background of Patients

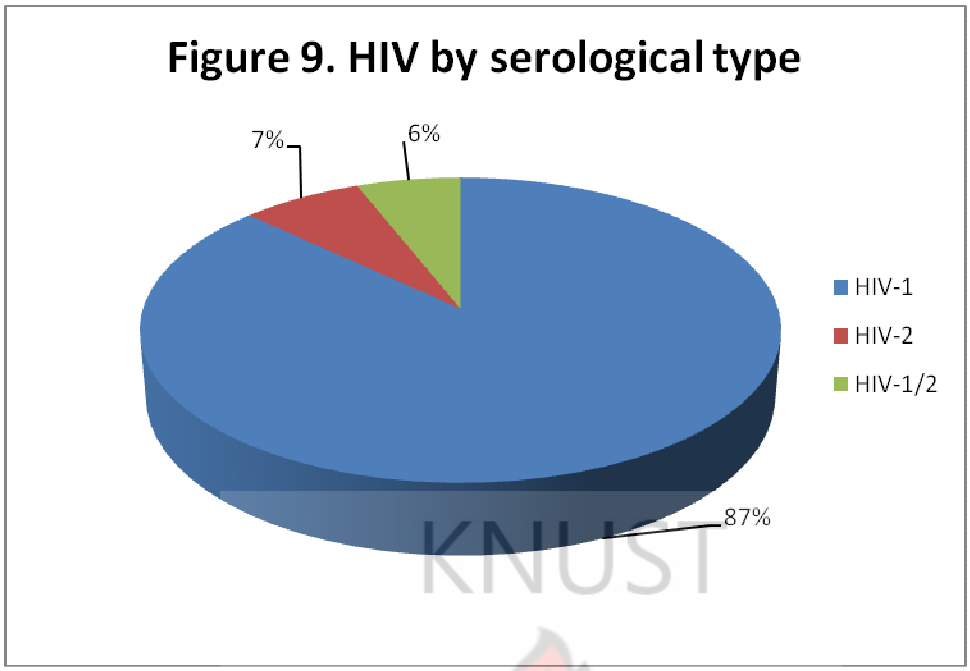
On religious beliefs, the majority of the patients were Christians (87.0%) and Muslims (7.4%). The remaining 5.6% were either members of traditional religion or other lesser known religions in Ghana (Fig. 8).

### 4.1.7 Patient Distribution

Finally, looking at the environment where patients lived, it was found that one hundred and one patients representing 93.5% were from urban centres whilst 7 patients (6.5%) were from rural areas (Data not shown).

### 4.2.0 HIV Prevalence and Types

The majority (87 %) of the patients were HIV-1 infected. This composed of 40 males and 56 females. Only 7% (4 males and 5 females) were HIV-2 infected. 6% (4 males and 3 females) had HIV-1 & 2 dual infections (Fig 9).






### 4.2.1 Patients and Drug Combination

All the HIV-1 patients were on either two NRTI (Stavudine + Lamivudine or Lamivudine + Zidovudine (Combivir) combined with a NNRTI (Nevirapine or Efavarenz).

In the case of the HIV-2 infected patients, 50% were on NNRTI (Efavarenz or Nevirapine) while the others were on PI (Abacavir, Nelfinavir or Kaletra)

In the case of HIV-1 & 2 patients however, 67% were on NNRTI (Nevirapine or Efavarenz) combined with Combivir.

### 4.3.0 CD4 Kinetics

There was a remarkable variation in response to treatment by all categories of patients with a general increase in trends of CD4 count in all three HIV types (Table 3)

Table 3. Mean CD4 counts of patients at various stages of therapy

| | **Mean CD4 count** | | |
|---|---|---|---|
| **Stage of Therapy** | **HIV-1** | **HIV-2** | **HIV-1/2** |
| **Pre therapy** | 205.22 | 316.86 | 161.00 |
| **1st post therapy** | 335.45 | 361.43 | 171.33 |
| **2nd post therapy** | 367.44 | 340.57 | 307.17 |
| **3rd post therapy** | 398.33 | 440.71 | 204.33 |
| **4th post therapy** | 438.48 | 396.80 | 259.50 |



The baseline mean CD4 counts for HIV-1, HIV-2 and HIV-1/2 were 205.22, 316.86 and 161.00 cell/ul respectively. By the fourth post therapy count, CD4 count for the various HIV types had risen to 438.48 for HIV-1, 396.80 for HIV-2 and 259.50 cells/ul for HIV-1/2 as shown in table 3.

### 4.3.1 Comparing HIV-1, HIV-2 and HIV-1/2

Table 2 further shows that there was an appreciable increase in patients mean CD4 cell count within the first 6 months of treatment in HIV-1, HIV-2 and HIV-1 & 2 patients. However, while the mean CD4 cells constantly increased in HIV-1 patients, it dropped twice, though insignificantly, at 12 and 24 months after therapy in HIV-2 patients and once at 24 months after therapy in the HIV-1&2 dually infected patients.

The table further shows that the baseline value of CD4 cells was significantly higher in HIV-2 infected patients (316.86 cells/ul) as against that of HIV-1 infected patients (205.22 cells/ul) and HIV-1/2 infected patients (161.00 cells/ul).

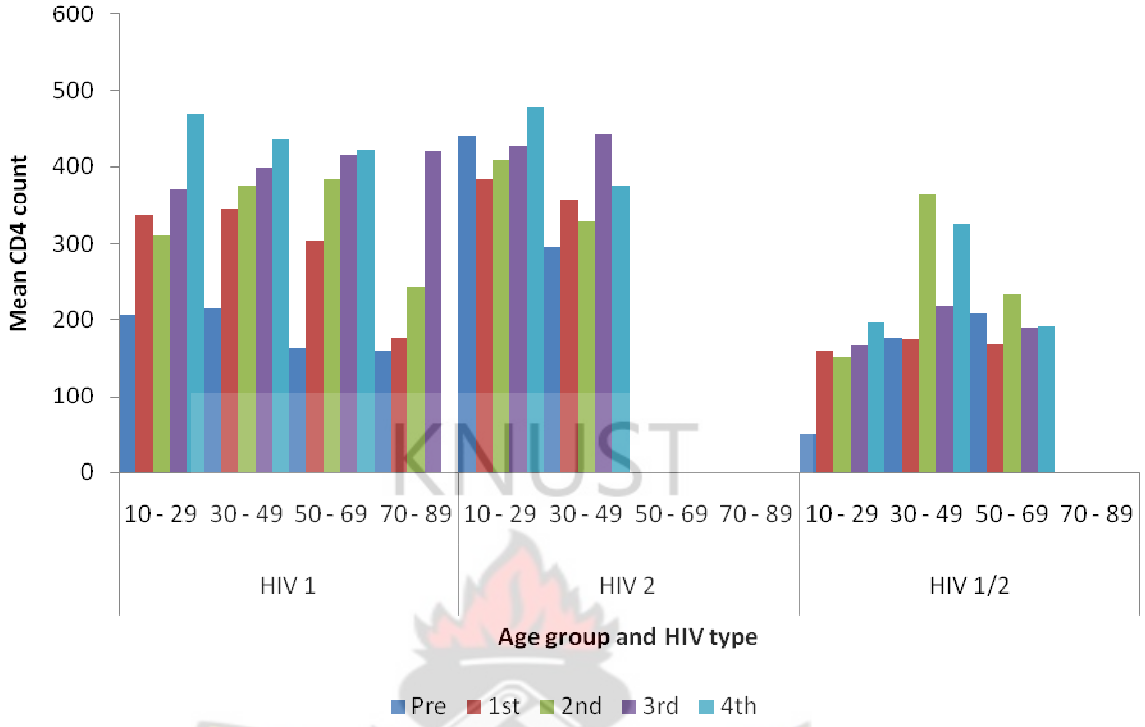
There was a significant statistical difference in the rate of increase in mean CD4 count for the three HIV types with a p-value of 0.0095 at 95% CI (Appendix 10). However, there was no significant difference between the rate of increase in mean CD4 count for HIV-1 and either HIV-2 or HIV-1 & 2 patients.



### 4.3.2 Immune response by Age

To determine whether patients of different age groups and different HIV types respond differently to antiretroviral therapy, it was realized that all the age groups in HIV-1 & 2 patients generally responded slowly to treatment as compared to either HIV-1 alone or HIV-2 alone where there was no marked difference in the rate of increase in mean CD4 cell count (Fig. 10). There were no subjects within the age group 50-69 and 70-89 for HIV-2 and 70-89 for HIV-1 & 2. As confirmed by statistical analysis, there was no significant difference in immune response of the different age groups from the various HIV types (P-value = 0.679 at 95% CI).

**Figure 10. Immune response by age**

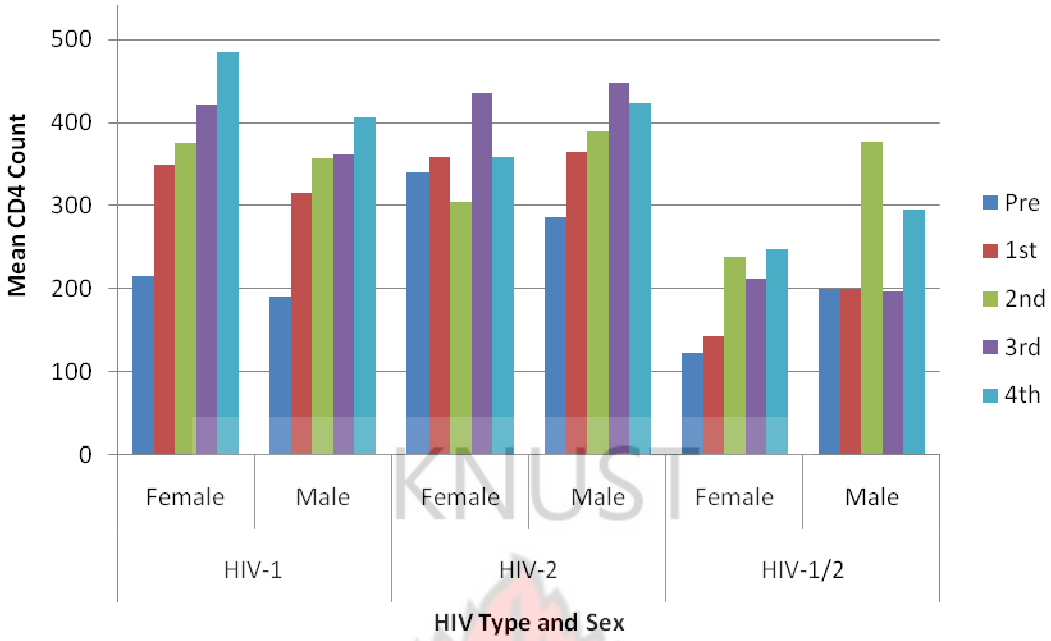





### 4.3.3 Immune response by sex

Considering whether gender difference affected patients' response to therapy, it was realized that there was no difference in response to therapy by gender within the three HIV types. However it was evident that both sexes with dual HIV-1 & 2 infections generally started with lower CD4 count and had lower recovery rate as compared to male and female patients with either HIV-1 and or HIV-2 alone who started with higher CD4counts with an appreciable CD4 recovery rate (figure 11). There was no statistical difference in the rate of increase in CD4 count of males compared to females (p-value =0.207 at 95% CI).

**Figure 11. Immune response by sex**



# CHAPTER FIVE

## Discussion

The study was aimed at monitoring how people infected with either HIV-2 or both HIV-1 & 2 would respond to ART. The rational was that the drugs used in the ART programme are specifically designed to treat patients infected with HIV-1. Although both HIV type-1 and type-2 are the main strains of lentivirus of the retrovirinae family that affect humans, there exists a huge homologous difference between them as they are only about 30% homologous. These imply that there may be a huge genetic difference between them which may affect their replicative activities.

These two viruses are also believed to be the causative agents of AIDS affecting human beings. Considering the genetic difference between them, one would expect that a drug that has been designed or developed to act by enzymatic activity would have effect on the type that are enzymatic specific, in this case against HIV type-1 and not type-2. Yet in this country and other places, people who are infected with HIV-2 are also put on these same drugs. The question therefore is how HIV-2 infected patients would respond to therapy that has been specifically designed against HIV-1.

To answer this question we looked at CD4 kinetics of HIV-2 infected patients on therapy and compared with those of HIV-1 and HIV-1 & 2. We specifically observed people undergoing ART with either HIV-2 alone infection or HIV-1 & 2 dual infection and compared their CD4 T-cell kinetics with that of those on ART with only HIV-1 alone infection.

The study revealed that whilst as many as 95 of the people made up of 38 males and 57 females were on ART with HIV-1 infection, only 7(3 males and 4 females) had

HIV-2. Six (3 males and 3 females) with HIV-1 & 2 co-infection were on ART, thus given an approximate ratio of 16:1:1 for HIV-1: HIV-2: HIV-1& 2 respectively.

This shows that contrary to the belief that HIV-2 is prevalent in West African countries (Kanki et al, 1999) including Ghana; it is not in dominant circulation. It also negates the belief that prevalent HIV-2 interferes and limits HIV-1 in circulation (Garret et al, 1992). Matsuda et al, 1993).

Our study again revealed that while HIV-1 infected people are likely to initiate ART with CD4 T-cell less than 250cells, HIV-2 infected patients are more likely to initiate therapy with CD4 cells more than 250 (average: 316.86 cells/ul)(Table 3). This suggest that people with HIV-2 infection are mostly put on treatment based on their clinical conditions other than their immune status as defined by their CD4 numbers. If this were to be so, then this would raise questions on the pathogencity of HIV-2, which of course can only be explained when one knows for how long the patients on ART had had their HIV infections.

But for the number of HIV-2 infected patients and the differences in virus genome, this study would have also offered the opportunity to study differences in HIV-infected patients initiating therapy with low or high CD4-cell numbers.

It was also observed that people infected with HIV-1 and initiating ART were more likely to be females with low education, low income earners and within their youthful age (average age 40 years).

From this observation, we think that women in their youthful age are vulnerable to HIV infection. We think this could be due to socio economic reasons. Perhaps being people with low education and low economic background, men may be taking advantage of them and demand sexual favors. In addition to this, their men will be

taking advantage of them and cheat on them by flirting around which may have resulted in their being infected and consequently giving the infection to their wives who may have no bargaining power because of their low social status. Whichever way it is, we think that HIV transmission is greatly influenced by poverty which in turn is influenced by educational level, socio-economic status and stigmatization

## LIMITATIONS

All serologically typed HIV-1 & 2 patients were assumed to be dually infected in this present study. There is therefore the likelihood of a level of biasness in the typing since serological typing may vary with molecular typing for the same blood specimen (Ficshetti et al, 2004).

## CONCLUSION AND RECOMMENDATIONS

From this study we could see that patients infected with HIV-2 comparably responded favorably to therapy just like those with HIV-1, despite being put on NNRTI. The few non-significant differences seen could be due to possibly non adherence to therapy of the affected patients.

The generally lower response of patients with HIV-1 & 2 dual infections is probably due to the combined effect or the synergistic effect of the two strains of HIV virus exerting a stronger inhibitory effect on the drugs.

Therefore, we think that the action of these drugs may be more on host factors other than virus-specific factors. That is why the differences in viral genome not withstanding HIV type 2, infected patients responded equally well to the supposed HIV-1 designed drugs.

This study has shown that HIV-2 patients at KATH responded well to available ARV's without any untoward complications, it is therefore recommended that patients with HIV-2 infection could continue to take their drugs without any fear of possible treatment failure.

## REFERENCES


1. Ameisen JC, Capron A. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. Immunol Today 1991; 12(4):102-5.
2. Bahrens GM, Stoll M, Schmidt RE. Lipodystrophy syndrome in HIV infection: What is it, what causes it and how can it be managed? Dong Saf 2000; 23:57-76.
3. Banda NK, Bernier J, Kurahara DK, et al. Cross-linking CD4 by HIV gp120 primes T cell for activation induced apoptosis. J Exp Med 1992, 176:1099-106.
4. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, et al (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science, 220:868-71.
5. Blanche S, Mayaux MJ, Rouzioux C, Teglas JP, et al. Relation of the course of HIV infection in children to the severity of the disease in their mothers at delivery. N Engl J Med 1994; 330(5):308-12.
6. Bonnet MM, Pinoges LLP, Varaine FFV, et al. Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden. AIDS 2006; 20:1275-79.
7. Branson BM, Point-of-Care Rapid Test for HIV Antibodies. J Lab Med 2003; 27:288-295.
8. Broder S, Gallo RC, (1984). A pathogenic retrovirus (HTLV-III) Linked to AIDS. N Engl J Med;311:1292 –

9. Brust S, Duttmann H, Feldner J, Gurtler L, Thorstensson R, Simon F. Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV-1/2/0 screening test. J Virol Meth 2000; 90:135-65.
10. Bylund DJ, Ziegner UT, Hopper DG. Review of testing for Human immunodeficiency virus. Clin Lab Med 1992; 12:305-33.
11. Cao Y, Qin L, Zhang L, Safrit J, Ho DD. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type-1 infection. N Engl J Med 1995; 332(4); 201-8.
12. Carlsen W. Quest for the origin of AIDS. Controversial book spurs search for how the worldwide search for the scourge of HIV began. San Francisco Chronicle, Sunday January 14th 2001: P. A1, A1 4-5.
13. Carr A, Cooper DA. Adverse effects of antiretroviral therapy. Lancet 2001; 356:1423-30.
14. Centers for Disease control. Revision of case definition of acquired immunodeficiency syndrome for national reporting- United States. MMWR Morb Mortal Wkly rep 1985; 34:373-375.
15. Chakrabarti S, panda S, Chatterjee A, Sarkar S, Manna B. et al, 2000. HIV-1 subtypes in injecting drug users and their non-injecting wives I Manipur, India. Indian J Med Res; 111: 189-94.
16. Chang LW, Osei-Kwasi M, Boakye D, Aidoo S, Hagy A, Curran JW, Vermund SH. 2002. HIV-1 and HIV-2 seroprevalence and risk factors among hospital outpatients in the Eastern Region of Ghana, West Africa. Acquir Immune Defic Syndr 29; 511-516.

17. Clark SJ, Saag MS, Decker WD,Campbell-Hill S, et al. High titres of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med 1991; 324 (14); 954-60.
18. Claval F, Guetard D, Brun-Vezinet F, Chamarat S, Rey MA, Santos-Ferreira O. Isolation of a new human retrovirus from West African patients with AIDS. Science 1986; 233: 343-346.
19. Cocchi F, DeVico AL, Garzino-Demo A, Arya S, Gallo RC, Lusso P. Identification of RANTES, MIP-1a, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. Science 1995, 270: 1811-5.
20. Cornelissen M, van Den Burg R, Zorgdrager F, Goudsmit J. 2000. Spresd of distinct human immunodeficiency virus type 1 AG recombinant lineages in Africa. J Gen Virol 81:515-523.
21. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, et al**.** Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med. 1994; 331:1173-80. [PMID: 7935654].
22. Coombs RW, Collier AC, Allain JP, Nikora B, et al. Plasma viremia in human immunodeficiency virus infection. N Engl J Med 1989;321(24):1626-31
23. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med 1991; 324(14):961-4.

24. Dalgleish AG, Beverley PC, Clapham PR, et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 1984, 312: 763-7.

25. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996, 381: 661-6.

26. De Jesus E, Herrera G, Teofilo E, et al. Abacavir versus zidovudine combined with lamivudine and efavirenz for the treatment of antiretroviral-naïve HIV-infected adults. Clin Infect Dis 2004;39:1038-46.

27. Deng HK, Unutmaz D, kewalramani VN, Littman DR. Expression cloning of new receptors used by simian and human immunodeficiency virus. Nature 1997, 388; 296-300.

28. Dicko M, Oni AQ, Ganivet S, Kone LP, Jacquet B. Safety of immunization injections in Africa: Not simply a problem of logistics. Bull World Health Organ 2000; 78:163-9.

29. Edwards TG, Hoffman TL, Baribaud F, et al. Relationships between CD4 independence, neutralization sensitivity and exposure of a CD4-induced epitope in an HIV-1 envelope protein. J Virol 2001, 75:5230-9.

30. Embretson J, Zapancic M, Ribas JL, Burke A, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993; 362 (6418):359-62.

31. European Collaborative Study. Risk factors for mother-to-child transmission of HIV-1. Lancet 1992;339:1007-12

32. Evans AS. The clinical illness promotion factor: a third ingredient. Yale J Biol Med 1982; 55(3-4):193-9.

33. Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science 1993a; 262 (3136):1011-8.

34. Finkel TH, Tudor-Williams G, Banda NK, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nature Medicine 1995; 1(2):129-34.

35. Fischetti L, Opare-Sem O, Candotti D, Sarkodie F. et al. Molecular epidemiology of HIV in Ghana: Dominance of CRF02_AG. J. Med. Virol 73:158-166, 2004.

36. Fox CH, Kotler D, Tierney A, Wilson CS, Fauci AS. Detection of HIV-1 RNA in the lamina propria of patients with AIDS and gastrointestinal disease. J Infect Dis 1989; 159(3):467-71.

37. Frimpong EH, Lawn P, Dwemoh B, Afful B, Acheampong JW. HIV infection in tuberculosis patients in Kumasi, Ghana. Ghana Med J 1997; 31b:850-854.

38. Gallo RC, Sarin PS, Gelmann EP, et al. Isolation of human t cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983, 220 ; 865-7.

39. Garret ED, Cullen BR, (1992) Comparative analysis of Rev functions in human immunodeficiency virus types 1 and 2. J Virol 66:4288-4294.

40. Gelderblom HR, Gentile M, Scheidler A, Özel M, Pauli G. Zur Struktur und Funktion bei HIV. AIFO 1993, 5: 231.

41. Gentilini M, Chieze F. (1990) Socioeconomic aspects of human immunodeficiency virus infection in developing countries. PMID 174(8):1209-19.
42. Giles RE, Perry KR, Parry JV. Simple/Rapid test devices for anti-HIV screening: Do they come up to the mark? J Med Virol 1999; 59:104-0.
43. Gregory M, John P, Keith F. et al. HIV- Related Stigma and Knowlwdge in the United States; Prevalence and trends, 1991-1999. Am J Public Health. 2002; 92:371-377.
44. Guidelines For Antiretroviral Therapy In Ghana, 2005. GHS/MOH pgs. 12, 32, 33.
45. Gurtler L. Difficulties and strategies of HIV diagnosis. Lancet 1996; 348:176-9.
46. Ho DD, Neuman AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV infection. Nature 1995; 373:123-126.
47. Hooper E. The race to conquer polio: early research and inactivated polio vaccine. Oral polio vaccine. In The River: A journey back to the source of HIV and AIDS. Penguin Books, London, UK. 1999. P. 194-217.
48. Hughes MD, Johnson VA, Hirsch MS, Bremer JW, Elbeik T, Erice A, et al. Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. ACTG 241 protocol Virology Sub-study Team. Ann Intern med 1997; 126:929-938.
49. Hugin AW, Vacchio MS, Morse HC III. A virus-encoded super antigen in a retrovirus-induced immunodeficiency syndrome of mice. Science 1991;252(5004):424-7

50. Hutto C, Parks WP, Lai SH, Mastrucci MT, et al. A hospital-based prospective study of perinatal infection with human immunodeficiency virus type 1. J Pediatr 1991; 118(3):347-53.

51. Janeway C. Immune recognition. Mls: makes little sense. Nature 1991;349(6309):459-61

52. Kanki PJ, Human immunodeficiency virus type-2 (HIV-2). AIDS Rev 1999; 1; 101-8.

53. Katrak SM. The origin of HIV and AIDS: An enigma of evolution. Ann Indiwn Acad Neurol 2006; 9:5-10.

54. Katzenstein DA, Hammer SM, Hughes MD, et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV infected adults with 200-500 CD4 cell per cubic millimeter. N Engl J Med 1996; 335:1091-1098.

55. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 1984, 312: 767-8.

56. Kostrikis LG, Badades E, Cao Y, Zhang L. et al, 1995. Genetic analysis of human immunodeficiency virus type-1 strain from patients from Cyprus; Identification of new designated subtype-1. J Virol 69(10):6122-30.

57. Lambert JS. Maternal and perinatal issues regarding HIV infection. Pediatr Ann 1990; 19(8):468-72.

58. Lawn SD, Bekker LG, Wood R. How effective does HAART restore immune responses to Mycobacterium tuberculosis? Implications for tuberculosis control. AIDS 2005a; 19:1113-24.

59. Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. Lancet Infect Dis 2005b; 5:361-73.

60. Leitner T. (1996) genetic variation of HIV-1; Molecular epidemiology and viral evolution. Swidish Institute for Infectious disease Control. Karolinska Institute Stockholm, Sweden.

61. Lifson JD, Reyes GR, McGrath MS, Stein BS, Engleman EG. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. Science 1986; 232(4754):1123-7.

62. Liao F, Alkhatib G, Peden KWC, Sharma G, Berger EA, Farber JM. STRL – 33,a novel chemokine receptor-like macrophage-tropic and T cell line-tropic HIV-1. J Exp Med 1997, 185; 2015-23. http;//amedeo.com/lit.php?id = 9166430

63. Lindgren S, Anzen B, Bohlin AB, Lidman K. HIV and child-bearing: clinical outcome and aspects of mother-to-infant transmission. AIDS 1991; 5(9):1111-6.

64. Lyerly HK, Matthews TJ, Langlois AJ, Bolognesi DP, Weinhold KJ. Human T-cell 59eropositive virus IIIB glycoprotein (gp120) bound to CD4 determinants on normal lymphocytes and expressed by infected cells serves as target for immune attack. Proc Natl Acad Sci USA 1987; 84(13):4601-5.

65. Mallon PWG, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1 infected men starting therapy. AIDS 2003; 17:971-79.

66. Mane P, Aggleton P. (2001) Gender and HIV/AIDS. What do men have to do with it? Current Sociology, 49(6), 23-27.

67. Marx PA, Alcabes PG, Drucker E. Serial human passage of Simian immunodeficiency virus by unsterile injections and the emergence of human immunodeficiency virus in Africa. Phil Trans R soc lon B 2001; 356:911-20.

68. Matsuda Z, Yu X, Yu QC, Lee TH, Essex M (1993) A virion-specific inhibitory molecule with therapeutic potential for human immunodeficiency virus type 1. Proc Natl Acad Sci USA 90:3544-3548.

69. Mellors JM, A. giorgi, J.V. Margolick, J.B. Tassoni, C.J. Gupta, P. Kingsley, L.A. Todd, J. A. Saah, A. J. Detals, R. Phair, J. P. Rinaldo, C.R., Jr. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med. 1997 Jun 15; 126(12):946-54.

70. Miyazaki M. (1995). Epidemiological characteristics of human immunodeficiency virus type-2 infection in Africa. Int J STD AIDS; 6:75-80.

71. Moore J. The puzzling origin of AIDS. Am Sci 2004; 92:540-7.

72. Muro-Cacho CA, Pantaleo G, Fauci AS. Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. J Immunol 1995; 154(10):5555-66.

73. Newell ML, Loveday C, Dunn D, Kaye S, Tedder R, et al. Use of polymerase chain reaction and quantitative antibody tests in children born to HIV-1 infected mothers. J Med Virol 1995; 47:330-5.

74. Oh SY, Cruickshank WW, Raina J, et al. Identification of HIV-1 envelope glycoprotein in the serum of AIDS and ARC patients. J Acquired Immune Defic Syndr 1992, 5: 251.

75. Ortiz M, Munoz L, Bernal A, Rodriguez A, Vadillo J, Salas A, Moreno A, Garcia-Saiz A. 2000. Molecular characterization of non-B HIV type 1subtypes from Africans in Spain. AIDS Res Hum Retroviruses 16(18):1967-1971.

76. Palella Fj jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, satten GA, Aschman DJ, Holmberg SD; Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.N Eng/Jmed 1998, 338(13):853-860.

77. Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993a; 328(5):327-35.

78. Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. N Eng J Med 1995a; 332:209-16.

79. Quarterly Technical bulletin on HIV/AIDS-STI's In Ghana. NACP/GHS 2005, pg.5

80. Republic of Ghana; Guidelines for Antiretroviral Therapy In Ghana. National HIV/AIDS/STI Control Programme, Ministry of Health/ Ghana Health Services, September 2005.

81. Requejo HIZ (2006). Worldwide molecular epidemiology of HIV. Rev Saude Publica 40(2).

82. Schiipbach J. Human Immunodeficiency Virus. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RM, Eds. Manual of Clinical Microbiology. 8th ed, ASM. Press, Washington 2003, 1253-81.

83. Schnittman SM, Denning SM, Greenhouse JJ, Justement JS, et al. Evidence for susceptibility of intrathymic T-cell precursors and their progeny carrying T-cell antigen receptor phenotypes TCR alpha beta + and TCR gamma delta + to human immunodeficiency virus infection: a mechanism for CD4+ (T4) lymphocyte depletion. Proc Natl Acad Sci USA 1990b; 87(19):7727-31.

84. Simon F, Matheron S, Tamalet C, et al. Cellular and plasma viral load in patients infected with HIV-2. AIDS 1993; 7:1411-7.

85. Sodroski J, Goh WC, Rosen C, Campbell K, Haseltine WA. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature 1986; 322(6078):470-4.

86. Stanley SK, Kessler SW, Justement JS, Schnittman SM, et al. CD34+ bone marrow cells are infected with HIV in a subset of 62eropositive individuals. J Immunol 1992; 149(2):689-97.

87. Stenson AL, Charalambous S, Dwadwa T. et al. (2005) Evaluation of antiretroviral therapy (ART) –related counseling in a workplace-based ART implementation programme, South Africa. AIDS CARE Vol 17(8); 949-957.

88. Sunila I, Vaccarezza M, Pantaleo G, Fauci AS, Orenstein JM. Gp120 is present on the plasma membrane of apoptotic CD4 cells prepared from

lymph nodes of HIV-1-infected individuals: an immune electron microscopic study. AIDS 1997, 11: 27-32

89. Sutherland R, Yang H, Scriba P, et al. Impaired IFN-g sectering capacity in mycobacterial antigen-specific CD4 T cells during chronic HIV-1 infection despite long term HAART. AIDS 2006; 20:821-29.
90. Tamashiro H, Maskill W, Emmanuel J, Franquex A, Sato P, Heymann D. Reducing the cost of HIV antibody testing. Lancet 1993; 342:87-90.
91. Terai C, Kornbluth RS, Pauza CD, Richman DD, Carson DA. Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1. J Clin Invest 1991;87(5):1710-5
92. Tindall B, Cooper DA. Primary HIV infection: host responses and intervention strategies. AIDS 1991; 5(1):1-14.
93. Vergis EN, Mellors JW (2000) Natural history of HIV-1 infection. Infection Dis Clin North Am. 14:809-825.
94. Wei X, Ghosh SK, Tailor ME, et al. Viral dynamics in human immunodeficiency virus type-1 infection. Nature 1995; 373:117-126.
95. Wei X, Ghosh SK, Taylor ME, Johnson VA, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995; 373:117-22.
96. Whittle H, Morris J, Todd J; et al. HIV-2 infected patients survive longer than HIV-1-infected patients. AIDS 1999; 8:1617-20.
97. WHO Report 2006: Global tuberculosis control – surveillance, planning, financing. Geneva, 2006 (WHO/HTM/TB/2006.362)

98. WHO. Scaling up antiretroviral therapy in resource- limited settings: treatment guidance for public health approach.2003 Revision. Geneva, Switzerland: World Health organization; 2004

99. WHO (World Health Organization) Rapid HIV tests: Guidelines for use in HIV testing and counseling services in resource-constrained settings. Geneva 2004.

100. Wolfe ND, Switzer WM, Carr JK, Bhullar VB, et al. Naturally acquired Simian retrovirus infections in central African hunters. Lancet 2004; 363:932-7.

101. Wong-Staal F. HIVes and their replication. In: Fundamental Virology, Ed.: Fields BN, Knipe DM et al. Raven Press, Ltd., New York 1991.

102. Yilmaz G. Diagnosis of HIV infection and laboratory monitoring of its therapy. J Clin Virol 2001; 21:187-96.

## APPENDIX 1- RAW DATA OF STUDIED POPULATION

HIV TYPE -1

| Code | Age | Sex | Marital Status | Educational level | Religion | Econ/ status | Support | Res | Pre therapy | 1st Post therapy | 2nd Post therapy | 3rd Post therapy | 4th Post therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC2/04/201 | 49 | F | W | T | C | H | C | U | 149 | 195 | 297 | 304 | 402 |
| CC2/04/060 | 55 | F | M | 1ST | C | L | C | U | 360 | 266 | 296 | 417 | |
| CC2/04/196 | 38 | F | S | 1ST | C | L | C | U | 171 | 343 | 422 | 323 | 365 |
| CC2/04/118 | 57 | F | W | 1ST | C | L | C | U | 419 | 195 | 481 | | |
| CC2/04/178 | 65 | F | W | 1ST | C | L | C | U | 121 | 218 | 167 | 168 | 186 |
| CC2/04/093 | 45 | M | M | 1ST | C | L | C | U | 120 | 666 | 552 | 623 | 549 |
| CC2/04/187 | 33 | M | S | 1ST | C | L | C | U | 218 | 432 | 688 | 672 | 728 |
| CC2/04/044 | 40 | F | M | 2ND | C | L | C | U | 181 | 432 | 566 | | |
| CC2/03/018 | 40 | F | D | N | C | L | C | U | 344 | 355 | 346 | 385 | |
| CC2/04/014 | 38 | F | S | N | C | L | C | U | 430 | 609 | 868 | 841 | 942 |
| CC2/03/015 | 42 | F | W | N | C | L | C | U | 238 | 325 | 120 | 284 | |
| CC2/04/192 | 60 | F | W | N | C | L | C | U | 198 | 365 | 674 | 873 | 798 |
| CC2/04/198 | 37 | F | D | N | M | L | C | U | 179 | 564 | 485 | 593 | 551 |
| CC2/04/061 | 26 | F | D | 1ST | C | L | F | U | 95 | 264 | 291 | 338 | 306 |
| CC2/03/045 | 30 | F | D | 1ST | C | L | F | U | 261 | 274 | 490 | 739 | 768 |
| CC2/03/055 | 35 | F | D | N | C | L | F | U | 433 | 397 | 372 | 254 | |
| CC2/03/051 | 60 | M | D | 1ST | C | L | O | U | 53 | 249 | 230 | 215 | 296 |
| CC2/03/051 | 60 | M | D | 1ST | C | L | O | U | 52 | 271 | 202 | 249 | 230 |
| CC2/04/124 | 44 | M | W | 1ST | C | L | O | U | 247 | 452 | 467 | 480 | 473 |
| CC2/04/052 | 42 | F | MI | N | M | L | O | U | 424 | 506 | 529 | 392 | |
| CC2/04/073 | 45 | F | D | N | O | L | O | R | 200 | 372 | 374 | 601 | |
| CC2/04/070 | 28 | F | MI | N | O | L | O | R | 431 | 393 | 298 | 356 | |
| CC2/04/063 | 47 | M | MI | N | O | L | O | U | 6 | 17 | 69 | 256 | |
| CC2/04/141 | 30 | M | S | T | C | H | P | U | 266 | 277 | 337 | 363 | 470 |
| CC2/04/179 | 25 | F | D | 1ST | C | L | P | U | 201 | 459 | 546 | 506 | 681 |
| CC2/03/052 | 35 | F | D | 1ST | C | L | P | U | 190 | 490 | 527 | 439 | 542 |
| CC2/04/026 | 37 | F | D | 1ST | C | L | P | U | 388 | 343 | 335 | 376 | 267 |
| CC2/04/155 | 37 | F | D | 1ST | C | L | P | U | 85 | 307 | 352 | 393 | 458 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC2/04/062 | 44 | F | D | 1ST | C | L | P | U | 515 | 674 | 9 | | |
| CC2/04/138 | 54 | F | M | 1ST | C | L | P | U | 7 | 173 | 217 | 538 | 694 |
| CC2/04/114 | 27 | F | S | 1ST | C | L | P | U | 226 | 528 | 646 | 721 | 521 |
| CC2/04/057 | 33 | F | S | 1ST | C | L | P | U | 123 | 251 | 347 | 356 | 540 |
| CC2/04/111 | 34 | F | W | 1ST | C | L | P | U | 289 | 255 | 347 | 341 | |
| CC2/03/025 | 36 | F | W | 1ST | C | L | P | U | 186 | 249 | 235 | 402 | 308 |
| CC2/04/166 | 44 | F | W | 1ST | C | L | P | U | 252 | 348 | 483 | 466 | |
| CC2/04/035 | 36 | M | M | 1ST | C | L | P | U | 13 | 182 | 178 | | |
| CC2/04/121 | 40 | M | W | 1ST | C | L | P | U | 127 | 206 | 224 | 283 | 281 |
| CC2/04/126 | 35 | M | D | N | C | L | P | R | 122 | 15 | 130 | 3 | |
| CC2/04/132 | 34 | F | W | T | C | L | P | R | 241 | 355 | 442 | 316 | 386 |
| CC2/03/054 | 35 | F | D | N | M | L | P | U | 124 | 565 | 532 | 581 | 738 |
| CC2/04/017 | 20 | F | S | N | M | L | P | U | 12 | 325 | 500 | 636 | 607 |
| CC2/04/075 | 50 | F | M | 1ST | O | L | P | R | 220 | 537 | 654 | 627 | 509 |
| CC2/04/065 | 31 | F | D | 2ND | C | M | P | U | 199 | 384 | 485 | 433 | 426 |
| CC2/04/055 | 36 | F | D | T | C | M | P | U | 147 | 472 | 375 | 472 | 546 |
| CC2/03/010 | 19 | F | S | 1ST | O | S | P | U | 389 | 350 | 250 | 389 | |
| CC2/04/130 | 47 | M | W | T | C | H | SI | U | 69 | 98 | 270 | 217 | 262 |
| CC2/03/07 | 32 | F | D | 1ST | C | L | SI | U | 247 | 523 | 475 | 457 | 434 |
| CC2/04/108 | 45 | F | D | 1ST | C | L | SI | U | 139 | 507 | 561 | 565 | 528 |
| CC2/04/076 | 26 | F | M | 1ST | C | L | SI | U | 77 | 403 | 57 | 190 | 498 |
| CC2/04/056 | 46 | F | MI | 1ST | C | L | SI | U | 276 | 207 | 347 | 355 | 348 |
| CC2/04/056 | 46 | F | MI | 1ST | C | L | SI | U | 276 | 207 | 347 | 355 | 348 |
| CC2/04/043 | 37 | F | S | 1ST | C | L | SI | U | 213 | 647 | 479 | 506 | 388 |
| CC2/04/045 | 31 | F | W | 1ST | C | L | SI | U | 388 | 308 | 379 | 355 | |
| CC2/04/007 | 35 | F | W | 1ST | C | L | SI | U | 442 | 406 | 270 | 368 | |
| CC2/04/083 | 48 | F | w | 1ST | C | L | SI | U | 197 | 276 | 418 | 359 | 355 |
| CC2/04/129 | 44 | M | D | 1ST | C | L | SI | R | 36 | 230 | 209 | 134 | 156 |
| CC2/04/097 | 45 | M | M | 1ST | C | L | SI | U | 304 | 310 | 68 | 395 | 529 |
| CC2/04/003 | 42 | M | W | 1ST | C | L | SI | U | 215 | 587 | 776 | | |
| CC2/04/003 | 42 | M | W | 1ST | C | L | SI | U | 215 | 587 | 776 | 622 | |
| CC2/03/032 | 43 | M | W | 1ST | C | L | SI | U | 85 | 170 | 222 | 199 | |
| CC2/04/160 | 38 | M | D | 2ND | C | L | SI | U | 59 | 111 | 155 | 221 | 223 |
| CC2/03/050 | 48 | M | D | 2ND | C | L | SI | U | 122 | 270 | 99 | | |
| CC2/04/058 | 71 | M | M | 2ND | C | L | SI | U | 299 | 192 | 333 | 421 | |
| CC2/03/07 | 40 | F | W | N | C | L | SI | U | 3 | 155 | 255 | 213 | 251 |
| CC2/04/101 | 68 | M | W | N | C | L | SI | U | 170 | 380 | 437 | 416 | 332 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC2/04/021 | 33 | F | M | 1ST | M | L | SI | U | 253 | 266 | 158 | 244 | 52 |
| CC2/04/051 | 42 | M | S | 1ST | M | L | SI | U | 343 | 184 | 264 | | |
| CC2/03/028 | 45 | F | W | 2ND | M | L | SI | U | 145 | 345 | 410 | 402 | |
| CC2/04/158 | 32 | M | D | 1ST | C | M | SI | U | 143 | 114 | 192 | 196 | 151 |
| CC2/04/112 | 39 | F | W | 1ST | C | S | SI | U | 199 | 335 | 409 | 374 | 380 |
| CC2/04/113 | 40 | F | D | T | C | S | SI | U | 6 | 64 | 167 | 249 | 297 |
| CC2/04/054 | 52 | M | S | T | C | H | SP | U | 1 | 147 | 251 | 285 | 258 |
| CC2/04/090 | 33 | F | M | 1ST | C | L | SP | U | 11 | 40 | 9 | 82 | 229 |
| CC2/04/131 | 28 | F | S | 1ST | C | L | SP | U | 313 | 221 | 89 | 148 | 366 |
| CC2/03/021 | 32 | F | S | 1ST | C | L | SP | U | 68 | 757 | 609 | 584 | 839 |
| CC2/04/069 | 48 | F | W | 1ST | C | L | SP | U | 30 | 181 | 433 | 415 | |
| CC2/04/081 | 37 | M | D | 1ST | C | L | SP | U | 111 | 390 | 385 | 556 | 439 |
| CC2/04/180 | 44 | M | D | 1ST | C | L | SP | U | 142 | 309 | 326 | 237 | 350 |
| CC2/04/015 | 45 | M | D | 1ST | C | L | SP | U | 64 | 310 | 313 | 247 | 366 |
| CC2/04/188 | 33 | M | M | 1ST | C | L | SP | U | 170 | 122 | 169 | 143 | 228 |
| CC2/04/068 | 36 | M | M | 1ST | C | L | SP | U | 585 | 598 | 542 | 428 | |
| CC2/04/002 | 45 | M | M | 1ST | C | L | SP | U | 197 | 226 | 525 | 417 | |
| CC2/04/090 | 46 | M | M | 1ST | C | L | SP | U | 351 | 304 | 308 | 363 | |
| CC2/04/177 | 41 | M | S | 1ST | C | L | SP | U | 186 | 291 | 304 | 314 | 374 |
| CC2/04/079 | 72 | M | W | 1ST | C | L | SP | U | 18 | 160 | 154 | | |
| CC2/04/025 | 27 | F | M | 2ND | C | L | SP | U | 318 | 285 | 192 | 179 | 424 |
| CC2/04/115 | 28 | F | M | N | C | L | SP | U | 1 | 140 | 236 | 249 | 348 |
| CC2/04/041 | 53 | M | M | N | C | L | SP | U | 353 | 398 | 715 | | |
| CC2/04/049 | 42 | M | M | 1ST | M | L | SP | U | 99 | 511 | 676 | 533 | 650 |
| CC2/04/032 | 40 | F | M | 1ST | C | M | SP | U | 6 | 159 | 356 | 823 | 532 |
| CC2/03/047 | 52 | M | M | 1ST | C | M | SP | U | 89 | 379 | 339 | 392 | 556 |
| CC2/03/047 | 52 | M | W | 1ST | C | M | SP | U | 89 | 379 | 339 | 392 | 379 |
| CC2/04/024 | 40 | M | M | 2ND | C | M | SP | U | 241 | 300 | 423 | 500 | |

HIV TYPE- 2

| Code | Age | Sex | Marital Status | Educational level | Religion | Econ/ status | Support | Res | Pre therapy | 1st Post therapy | 2nd Post therapy | 3rd Post therapy | 4th Post therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC2/03/06 | 42 | M | W | 1ST | C | L | C | U | 178 | 350 | 433 | 440 | 430 |
| CC2/04/116 | 42 | M | M | 2ND | C | L | SI | U | 266 | 304 | 177 | 255 | 381 |
| CC2/03/07 | 29 | F | D | N | C | L | SI | U | 441 | 384 | 409 | 429 | 477 |
| CC2/04/018 | 43 | F | M | N | O | L | SI | U | 233 | 376 | 277 | 210 | 240 |
| CC2/04/013 | 46 | F | M | 2ND | C | L | SP | U | 300 | 506 | 286 | 806 | |
| CC2/04/004 | 41 | M | M | 1ST | C | M | SP | U | 415 | 442 | 558 | 649 | 456 |
| CC2/04/001 | 31 | F | M | 2ND | C | M | SP | U | 385 | 168 | 244 | 296 | |

HIV TYPE-1&2

| Patient Code | Age | Sex | Marital Status | Educational level | Religion | Econ/status | Support | Res | Pre therapy | 1st Post therapy | 2nd Post therapy | 3rd Post therapy | 4th Post therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC2/04/023 | 60 | F | D | N | C | L | C | U | 207 | 168 | 233 | 189 | 191 |
| CC2/04/140 | 49 | M | D | 1ST | C | M | SI | U | 83 | 117 | 120 | 60 | |
| CC2/03/026 | 42 | M | S | 1ST | C | L | SI | U | 359 | 170 | 645 | 218 | |
| CC2/04/122 | 29 | F | S | T | C | M | SI | R | 50 | 160 | 150 | 167 | 197 |
| CC2/03/07 | 44 | F | M | 1ST | C | L | SP | U | 111 | 102 | 331 | 281 | 355 |
| Cc2/03/049 | 33 | M | M | 1ST | C | L | SP | U | 156 | 311 | 364 | 311 | 295 |

**KEY**

Support: SI= Siblings, C =Child, P =Parent, F =Friend, SP =Spouse, O =Others (Uncle, Nephew, Aunt, etc

Economic status: L =Low, M =Medium, H =High, S =Student

Marrital status: M =Married, S =Single, D =Divorced, W =Widowed, MI =Missing

Eductional level: 1st = First cycle, 2nd =Second cycle, T =Tertiary

Residence: R= Rural, U= Urban, SU= Semi Urban

Religion: M=Moslem, C=Christian, O=Others (Traditional religion, Buddhist etc)

**Appendix 2- Materials Needed for Vironostika Assay**

Vironostika test kit.

Incubator

Micropipettes

Distilled water

Spectrophotometer

Specimen



Wash machine

Disposable gloves

Chlorine disinfectant

Measuring cylinders

**Appendix 3- Principle of Vironostika Assay**

Vironostika HIV Uni-Form II plus O ELISA operates on a one-step 'sandwich' principle. The microtitre wells within which the test is performed are coated with antigens of HIV-1, HIV-2 as well as HIV-1 group O. The conjugate is made of HIV antigens coupled to a horseradish peroxidase (HPR) while the substrate is basically tetramethylbenzidine (TMB) and peroxide.

Each microtitre well contains an HRP-labeled conjugate sphere of the same HIV-antigen mixture. The specimen diluent when added to the well dissolves the conjugate sphere. Then the test sample or the appropriate control containing the anti-HIV-1, anti-HIV-2 or anti-HIV-1 group O is incubated in the microtitre wells. There is the

formation of an antigen-anti-HIV-enzyme labeled antigen complex. Following a wash procedure, the TMB substrate is added and a blue colour develops which turns yellow when the reaction is stopped with sulfuric acid. The colour intensity is then converted into quantitative values by using a spectrophotometer at a wavelength of 450nm.

**Appendix 4- Principle of Rapitest HIV-1 & 2 (HIV Confirmatory Testing)**



This is a one step immunochromatographic rapid assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, and IgA) specific to HIV-1, including subtype O, and HIV-2 serologically and simultaneously in human serum, plasma or whole blood. The test device contains a membrane strip precoated with recombinant HIV-1 capture antigen gp41 including (subtype O, p24) on band 1 and with recombinant HIV-2 capture antigen (gp36) on band 2, respectively. The recombinant HIV-1/2 antigens gp41, p24 and gp36-colloid gold conjugate and serum samples move along the membrane chromatographically to the test region and forms visible bands marked '1' and '2' representing either HIV-1 or HIV-2 or both as HIV-1& 2 dually infected patients. The test device has a control band marked 'C' the appearance of which is an indication that the test device is working properly.

**Appendix 5- Materials Needed for CD4 Count Determination**

- The FACSCount automated machine
- FACSCount coring station – A device used to open the reagent and control tubes to prepare them for use.

- FACSCount electric pipette – An automated preprogrammed pipette to accurately deliver 50ul of fluid.
- A FACSCount work station.
- System fluid – A saline solution that flows through the fluid system.
- Thermal printer paper
- Waste reservoir
- Cleaning tubes and dispensing bottles – Test tubes and dispensing bottles provided for cleaning agents (chlorine bleach and distilled water)
- FACSCount reagent
- Controls – Four bead concentrations (Zero, low, medium and high)
- Pipette fin tips
- Vortex machine – Used for mixing samples
- K3 EDTA Vacutainer tubes for blood collection



**Appendix 6- Principle of Operation of Facscount**

The FACSCount operates on the principle of immuno fluorescence. A single test requires one convenient ready to use reagent tube pair; one tube determines the absolute number of helper/inducer T-lymphocytes (CD4/CD3), the other tube determines the absolute number of suppressor/cytotoxic T-lymphocytes (CD8/CD3). Both tubes measure the absolute number of total lymphocytes (CD3). Procedure requires minimal sample handling. When blood sample is added to the reagents, fluorochrome-lebeled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative has been added to the reagent tubes, the sample is

run on the instrument. On incidence of the laser light, the fluorochrome-labeled cells fluoresce and that allows for detection and quantification. The total T-lymphocyte cell count is then printed out immediately via an internal printer after each sample run.

**Appendix 7 - Case Report Form**

EFFECTS OF HIV-2 CO-INFECTION ON HIV-1&2 DUALLY INFECTED PATIENTS' RESPONSE TO HAART

Study ID: [ ] [ ] [ ] Code: _____

Name: _____

Age (years): [ ] [ ]

Sex

[0]-Male [1]-Female

Marital Status

[0]-Single [1]-Married [2]-Divorced [3]-Widowed

Educational Level

[0]-Primary School
[1]-Middle School/JSS
[2]-Secondary School/SSS
[3]-Tertiary
[4]-None (Illiterate)

Religion

[0]-Christian
[1]-Moslem
[2]-Other (Traditional, Hindis, Eckanker, Buddhist)

Occupation

[0]-Unemployed [1]-Trader [2]-Farmer [3]-Student [4]-Teacher
[5]-Civil Service [6]-Other

Other Occupation_____

Support or Monitor

[0]-Husband
[1]-Wife
[2]-Sibling
[3]-Daughter/Son
[4]-Parents
[5]-Other Other

Support/Monitor:_____

Residence: _____

HIV Type

[0]-Type I
[1]-Type II

[2]-Type I & II

**CD4 VALUES**

Pre-therapy:_____

1st Post Therapy: _____

2nd Post Therapy: _____

3rd Post Therapy: _____

4th Post Therapy: _____

**TREATMENT (**tick as appropriate)

NRTI

[0]-Stavudine(D4T)
[1]Lamivudine(3TC)
[2]Combivir(3TC+AZT)
[3] Zidovudine(AZT)
[4]Didanosine(ddl)
[5]Zalcitabine(ddc)
[6]Abacavir(ABC)

NRTI

[0]-Stavudine(D4T)
[1]Lamivudine(3TC)
[2]Combivir(3TC+AZT)
[3] Zidovudine(AZT)
[4]Didanosine(ddl)
[5]Zalcitabine(ddc)
[6]Abacavir(ABC)



NNRT

[0] Nevirapine(NVP)
[1] Efavirenz(EFV)

PI

[0]-Indinavir
[1] Ritonavir
[2] Nelfinavir(NFV)
[3] Kaletra

Regimen

[0]-First line Regimen
[1]-Second line Regimen

**Appendix 8 - Immune Response by Age**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 7 | 2589.72 | 369.96 | 9536.281 |
| Column 2 | 7 | 2450.53 | 350.07 | 5895.909 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| Between Groups | 1383.847 | 1 | 1383.847 | 0.17945 | 0.67928 | 4.74725 |
| Within Groups | 92593.14 | 12 | 7716.095 | | | |
| Total | 93976.99 | 13 | | | | |

**Appendix 9 - Immune Response by Sex**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| MALE | 7 | 2684.13 | 383.4471 | 6362.74 |
| FEMALE | 7 | 2262.77 | 323.2529 | 7900.214 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 12681.73 | 1 | 12681.73 | 1.778276 | 0.207122 | 4.747225 |
| Within Groups | 85577.72 | 12 | 7131.477 | | | |
| Total | 98259.46 | 13 | | | | |

**Appendix 10- Comparing Immune Response in HIV-1, HIV-2 and HIV1/2**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| HIV-1 | 5 | 1744.92 | 348.984 | 7909.111 |
| HIV-2 | 5 | 1856.37 | 371.274 | 2368.365 |
| HIV 1/2 | 5 | 1103.33 | 220.666 | 3812.99 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 66075.2 | 2 | 33037.6 | 7.034034 | 0.009516 | 3.885294 |
| Within Groups | 56361.86 | 12 | 4696.822 | | | |
| Total | 122437.1 | 14 | | | | |