VIROLOGICAL CHANGES IN HIV INFECTED PEOPLE UNDERGOING HERBAL TREATMENT

By

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A Thesis submitted to the Department of Clinical Microbiology
Kwame Nkrumah University of Science and Technology
In partial fulfilment of the requirements for the Degree

Of

MASTER OF SCIENCE
School of Medical Sciences

College of Health Sciences

November, 2009

DECLARATION

I hereby declare that this submission is my own work towards the award of an MSc. and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

In a study to assess the response of HIV/AIDS patients to some herbal products as HIV/AIDS therapeutic, we evaluated three of such products in four HIV-infected patients by looking at virological (viral load) and other parameters such as CD4+ T cell count, heamatology and biochemistry that may be altered in these patients over a period of six months.

Results obtained showed that two patients responded successfully to their treatments suggesting the potential potency of the herbal products they had taken. However, the remaining two patients showed evidence of virological and immunological treatment failure.

Finally, the achievement of health improvement within six months indicates that herbal medicine can be used as an alternative treatment for HIV/AIDS, and that it is a good immune booster and probable "virus-cidal" factor.

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ACKNOWLEDGEMENT

My greatest appreciation goes to the Lord Almighty for bringing me this far throughout my course. Next, I wish to convey sincere gratitude to Dr.T.B Kwofie for accepting me as his student. His support, guidance and valuable suggestions were of immense importance to this study.

Special thanks go to all the staff members of the Department of Microbiology, KNUST, and more specifically to Mr Feglo. This thesis also benefited greatly from equipments, suggestions and contributions by Mr Albert Dompreh and Mr Kwabena Adjei Asante staff of KATH Serology laboratory. I am also grateful to my project mates Maxwell Anderson and Gloria Kata.

Last but not the least, I would like to extend my special thanks to my family, all my friends especially Afrifa, Kwaku, Boadu, Chocho and Afoakwa. Your company, friendship, support and encouragement in times of need were invaluable. My heartfelt gratitude goes to Precious Barnes for her prayers and support. May God bless you all.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human Immunodeficiency Virus (HIV) is currently the most infectious pathogen with devastating consequences. In 2005, UNAIDS estimated that 40 million people were infected worldwide, with 25 million in sub-Saharan Africa, while approximately 2.4 million deaths were attributed to AIDS in 2005 (UNAIDS, 2005). In the early 1980s when the HIV/AIDS epidemic began, people with AIDS were not likely to live longer than a few years. AIDS is the most severe acceleration of infection with HIV. HIV is a retrovirus that primarily infects vital organs of the human immune system such as $CD4^+$ T cells (a subset of T cells), macrophages and dendritic cells. It directly and indirectly destroys $CD4^+$ T cells (Alimonti *et al*., 2003). Once HIV has killed so many $CD4^+$ T cells that there are fewer than 200 of these cells per microliter (µL) of blood, cellular immunity is lost. Acute HIV infection progresses over time to clinical latent HIV infection and then to early symptomatic HIV infection and later to AIDS, which is identified either on the basis of the amount of $CD4^+$ T cells remaining in the blood, and/or the presence of certain infections (ADDHD, 2003). With the development of safe and effective drugs, however, people infected with HIV now can live longer and lead healthier lives. To date HIV/AIDS has no cure. There has been a fervent and continuous search for preventive and curative therapeutics in an effort to stem the spread of the pandemic (McMichael and Hanke, 2003; Pomerantz and Horn, 2003).

The introduction of highly active antiretroviral therapy (HAART) into clinical practice in 1996 has dramatically changed the development of HIV-related diseases in industrialized countries (Bonfanti, 1999; Shafer, 1999; Tirelli, 2001; Vella, 2000). Although they cannot cure HIV infection and AIDS except its management, the antiretrovirals have an impact on reducing morbidity and mortality, suppressing viral load and reducing HIV transmission, prolonging lives, and improving the quality of life of many people living with HIV/AIDS (WHO, 2002). However, HAART has a limited response in some patients and is also associated with some drug toxicities. Moreover, many people with HIV/AIDS in developing countries cannot access HAART due to high cost of the drugs, limited human and structural resources, and social stigma. In addition, antiretroviral drugs (ARVs) require the support of expensive medical infrastructure not available in many developing countries and rarely available in rural areas of any developing country. ARVs can also produce significant side effects, as well as sometimes being ineffective. Adverse effects which include heart attacks, adult diabetes and other serious conditions (Yolan *et al*., 2007).

The high cost, unsatisfactory effect and unavailability of anti-HIV drugs to people living with AIDS in the developing world leads many to turn to herbal medicine to manage HIV related illness. Medicinal plants are popularly assumed to be a safe and natural alternative to conventional medications, On the other hand, there is an increasing number of reports in the medical literature about liver toxicity, kidney failure and other adverse events from some herbal products (Ishizaki, 1996; Melchart, 1999), which sometimes aggravate the already worsened conditions of those HIV/AIDS patients using them. However several studies have demonstrated the inhibitory properties of a variety

of crude plant extracts, as well as chemically characterized phytomolecules against different stages of the life cycle of HIV (Bessong, O. P. and Obi, C. L., 2006). Some of these studies focused on plant parts used traditionally in specific geographic locales in the treatment of various forms of infectious diseases. Interestingly, a few plant derived compounds such as papavarine glycyrrhizin and trichosanthin were seen to have promise and have been evaluated in AIDS patients (Bessong, O. P. and Obi, C. L., 2006).

In a study to clinically assess the efficacy of South African traditional medicine by monitoring viral load and CD4 counts, (Tshibangu *et al*., 2004) described an improvement in the immune system and general well-being of patients, due to increases in CD4+ T cell and decrease in viral load, when these markers were monitored for 12 months in HIV/AIDS participants. These developments show that useful anti-HIV agents could be obtained from plants sources (Vlietinck *et al*., 1998; De Clercq, 2000; Kong *et al*., 2003), as well as proves that herbal medicines might have the potential to alleviate symptoms, reduce viral load, and increase CD4+ cells for HIV-infected individuals and AIDS patients (Burack, 1996; Durant, 1998; Kang, 1999; Liu, 2000; Lu, 1993; Zheng, 1999).

Current treatment options for HIV/AIDS have not been satisfactory and the quest for effective curative or preventive therapies to treat HIV/AIDS is of paramount importance. This study is to assess the response of HIV/AIDS patients to some herbal products used to treat HIV/AIDS in Ghana. Specifically viral load and other parameters such as $CD4^+$ T cells, chemistry and haematology of study subjects were monitored. This was aimed at determining the response of HIV/AIDS patients to these supposed anti-HIV herbal

product in the control of HIV/AIDS or symptoms of the infection and therefore, can be recognized as a viable alternative for HIV/AIDS treatment and be made accessible to all.

1.2 Aims of the study

The aim of this study is therefore to determine the response of HIV/AIDS patients to some herbal products by evaluating virological (viral load) and other parameters such as $CD4^+$ T cells count, biochemistry and haematology. It is aimed to establish whether the herbal products can be used as viable alternative therapy for the treatment of HIV/AIDS.

1.3 Objectives

- To assess the response of HIV/AIDS patients to three herbal products.
- To assess the beneficial and harmful effects of some herbal products on patients with HIV/AIDS infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS)

Acquired Immune Deficiency Syndrome (**AIDS**) is a set of symptoms and infections resulting from the damage to the human immune system caused by the human immunodeficiency virus (HIV) (Weiss, 1993). This condition progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors. HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, preseminal fluid, and breast milk. This transmission can involve anal, vaginal or oral sex, blood transfusion, contaminated hypodermic-needles, exchange between mother and baby during pregnancy, childbirth, or breastfeeding, or other exposure to one of the above bodily fluids.

AIDS is now a pandemic (Kallings, 2008). In 2007, an estimated 33.2 million people lived with the disease worldwide, and it killed an estimated 2.1 million people, including 330,000 children (UNAIDS, WHO, 2007). Over three-quarters of these deaths occurred in sub-Saharan Africa, (UNAIDS, WHO. 2007) retarding economic growth and destroying human capital (Bell et al., 2008). Most researchers believe that HIV originated in sub-Saharan Africa during the twentieth century (Gao *et al*., 1999). AIDS was first recognized by the U.S. Centers for Disease Control and Prevention in 1981 and

its causative agent (HIV), identified by American and French scientists in the early 1980s (Gallo, 2006).

Although treatments for AIDS and HIV infection can slow the course of the disease, there is currently no vaccine or cure. Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but these drugs are expensive and routine access to antiretroviral medication is not available in all countries (Pelella, *et al*., 1998). Due to the difficulty in treating HIV infection, preventing infection is a key aim in controlling the AIDS epidemic, with health organizations promoting safe sex and needle-exchange programmes in attempts to slow the spread of the virus.

2.2 The clinical course of infection

Infection with HIV-1 is associated with a progressive decrease of the $CD4^+$ T cell count and an increase in viral load. The stage of infection can be determined by measuring the patient's $CD4^+$ T cell count, and the level of HIV in the blood.

HIV infection has basically four stages: incubation period, acute infection, latency stage and AIDS. The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks. The second stage, acute infection, which lasts an average of 28 days and can include symptoms such as fever, rash , myalgia lymphadenopathy (swollen lymph nodes), pharyngitis (sore throat), malaise and mouth and esophageal sores. The latency stage, which occurs third, shows few or no symptoms and can last anywhere from two weeks to twenty years and beyond. AIDS, the fourth and final stage of HIV infection shows as symptoms of various opportunistic infections.

2.2.1 Acute HIV infection

The initial infection with HIV generally occurs after transfer of body fluids from an infected person to an uninfected one. The first stage of infection, the primary, or acute infection, is a period of rapid viral replication that immediately follows the individual's exposure to HIV leading to an abundance of virus in the peripheral blood with levels of HIV commonly approaching several million viruses per mL (Piatak *et al*., 1993). This response is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells. This acute viremia is associated in virtually all patients with the activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ T cell counts rebound to around 800 cells per µL (the normal blood value is 1200 cells per µL). A good $CD8^+$ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo *et al*., 1997). During this period (usually 2-4 weeks post-exposure) most individuals (80 to 90%) develop an influenza or mononucleosis-like illness called acute HIV infection, the most common symptoms of which may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophagal sores, and may also include, but less commonly, headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms. Infected individuals may experience all, some, or none of these symptoms. The duration of symptoms varies, averaging 28 days and usually lasting at least a week (Kahn and Walker, 1998). Because of the nonspecific nature of these symptoms, they are often not recognized as signs of HIV infection. Even if patients go to their doctors or a hospital, they will often be misdiagnosed as having one of the more common infectious diseases

with the same symptoms. Consequently, these primary symptoms are not used to diagnose HIV infection as they do not develop in all cases and because many are caused by other more common diseases. However, recognizing the syndrome can be important because the patient is much more infectious during this period (Daar *et al*., 2001).

2.2.2 Latency stage

A strong immune defense reduces the number of viral particles in the blood stream, marking the start of the infection's clinical latency stage. Clinical latency can vary between two weeks and 20 years. During this early phase of infection, HIV is active within lymphoid organs, where large amounts of virus become trapped in the follicular dendritic cells (FDC) network (Burton *et al*., 2002). The surrounding tissues that are rich in $CD4^+$ T cells may also become infected, and viral particles accumulate both in infected cells and as free virus. Individuals who are in this phase are still infectious. During this time, $CD4^+$ $CD45RO^+$ T cells carry most of the proviral load (Clapham *et al*., 2001).

2.2.3 AIDS

When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and infections with a variety of opportunistic microbes appear. The first symptoms often include moderate and unexplained weight loss, recurring respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis), prostatitis, skin rashes, and oral ulcerations. Common opportunistic infections and tumors, most of which are normally controlled by robust $CD4^+$ T cell-mediated immunity then start to affect the patient. Typically, resistance is lost early on to oral

Candida species and to Mycobacterium tuberculosis, which leads to an increased susceptibility to oral candidiasis (thrush) and tuberculosis. Later, reactivation of latent herpes viruses may cause worsening recurrences of herpes simplex eruptions, shingles, Epstein-Barr virus-induced B-cell lymphomas, or Kaposi's sarcoma, a tumor of endothelial cells that occurs when HIV proteins such as Tat interact with Human Herpesvirus-8. Pneumonia caused by the fungus Pneumocystis jirovecii is common and often fatal. In the final stages of AIDS, infection with cytomegalovirus (another herpes virus) or Mycobacterium avium complex is more prominent. Not all patients with AIDS get all these infections or tumors, and there are other tumors and infections that are less prominent but still significant.

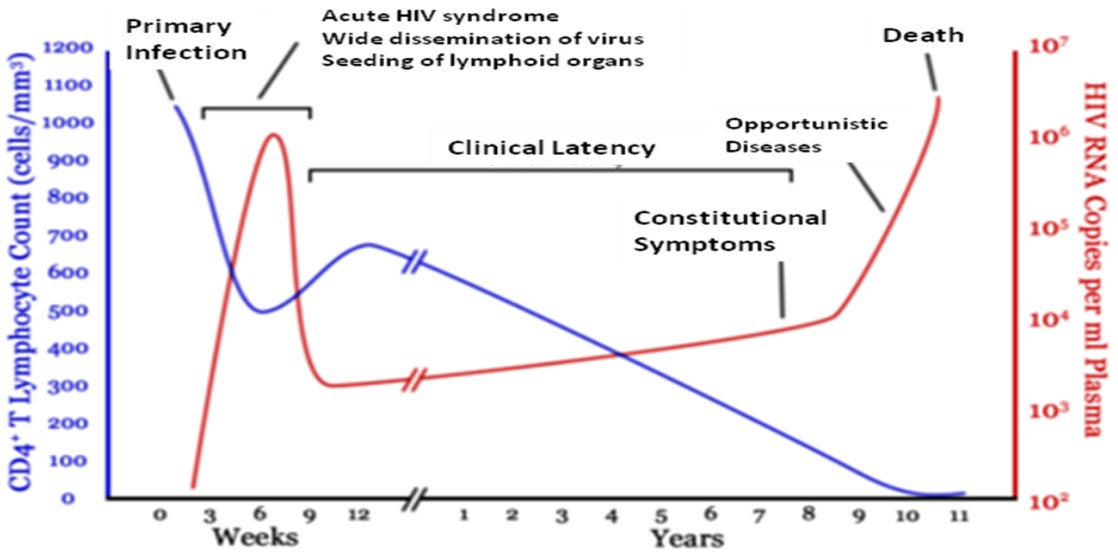

Figure 2.1: A generalized graph of the relationship between HIV copies (viral load) and CD4 counts over the average course of untreated HIV infection; any particular individual's disease course may vary considerably. $CD4^+$ T Lymphocyte count (cells/mm³). HIV RNA copies per mL of plasma (Wikipedia, 2008).

2.3 Symptoms

The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages. Opportunistic infections are common in people with AIDS (Holmes *et al.*, 2003). HIV affects nearly every organ system. People with AIDS also have an increased risk of developing various cancers such as Kaposi's sarcoma, cervical cancer and cancers of the immune system known as lymphomas. Additionally, people with AIDS often have systemic symptoms of infection like fevers, sweats (particularly at night), swollen glands, chills, weakness, and weight loss (Guss, 1994). The specific opportunistic infections that AIDS patients develop depend in part on the prevalence of these infections in the geographic area in which the patient lives.

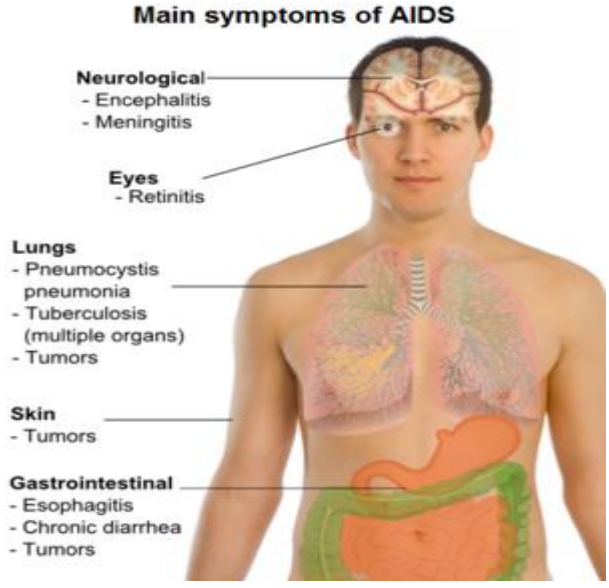

Figure 2.2: Shows the main symptoms of AIDS with respect to the organs involved. (Wikipedia, 2008).

2.3.1 Cause of HIV/AIDS disease

AIDS is the most severe acceleration of infection with HIV. HIV is a retrovirus that primarily infects vital organs of the human immune system such as $CD4^+$ T cells (a subset of T cells), macrophages and dendritic cells. It directly and indirectly destroys $CD4^+$ T cells (Alimonti *et al*., 2003). Once HIV has killed so many $CD4^+$ T cells that there are fewer than 200 of these cells per microliter (μL) of blood, cellular immunity is lost. Acute HIV infection progresses over time to clinical latent HIV infection and then to early symptomatic HIV infection and later to AIDS, which is identified either on the basis of the amount of $CD4^+$ T cells remaining in the blood, and/or the presence of certain infections (ADDHD, 2003) .

In the absence of antiretroviral therapy, the median time of progression from HIV infection to AIDS is nine to ten years, and the median survival time after developing AIDS is only 9.2 months (Morgan *et al*., 2002). However, the rate of clinical disease progression varies widely between individuals, from two weeks up to 20 years. Many factors affect the rate of progression. These include factors that influence the body's ability to defend against HIV such as the infected person's general immune function (Clerici *et al*., 1996; Morgan *et al*., 2002). Older people have weaker immune systems, and therefore have a greater risk of rapid disease progression than younger people. Poor access to health care and the existence of coexisting infections such as tuberculosis also may predispose people to faster disease progression (Morgan *et al*., 2002; Gendelman *et al*., 1986; Bentwich *et al*., 1995). The infected person's genetic inheritance plays an important role and some people are resistant to certain strains of HIV. An example of this is people with the homozygous CCR5-Δ32 variation are

resistant to infection with certain strains of HIV (Tang and Kaslow, 2003). HIV is genetically variable and exists as different strains, which cause different rates of clinical disease progression (Quinones *et al*., 1998; Campbell *et al*., 2004; Kaleebu *et al*., 2002).

2.3.2 Sexual transmission

Sexual transmission occurs with the contact between sexual secretions of one person with the rectal, genital or oral mucous membranes of another. Unprotected receptive sexual acts are riskier than unprotected insertive sexual acts, and the risk for transmitting HIV through unprotected anal intercourse is greater than the risk from vaginal intercourse or oral sex. However, oral sex is not entirely safe, as HIV can be transmitted through both insertive and receptive oral sex (Rothenberg *et al*., 1998; Mastro *et al*., 1996). Sexual assault greatly increases the risk of HIV transmission as protection is rarely employed and physical trauma to the vagina frequently occurs, facilitating the transmission of HIV (Koenig *et al*., 2004).

Other sexually transmitted infections (STI) increase the risk of HIV transmission and infection, because they cause the disruption of the normal epithelial barrier by genital ulceration and/or microulceration; and by accumulation of pools of HIV-susceptible or HIV-infected cells (lymphocytes and macrophages) in semen and vaginal secretions. Epidemiological studies from sub-Saharan Africa, Europe and North America suggest that genital ulcers, such as those caused by syphilis and/or chancroid, increase the risk of becoming infected with HIV by about four-fold. There is also a significant although lesser increase in risk from STIs such as gonorrhea, Chlamydial infection and trichomoniasis, which all cause local accumulations of lymphocytes and macrophages (Laga *et al*., 1991).

Transmission of HIV depends on the infectiousness of the index case and the susceptibility of the uninfected partner. Infectivity seems to vary during the course of illness and is not constant between individuals. An undetectable plasma viral load does not necessarily indicate a low viral load in the seminal liquid or genital secretions. However, each 10-fold increase in the level of HIV in the blood is associated with an 81% increased rate of HIV transmission (Laga *et al*., 1991; Tovanabutra *et al*., 2002). Women are more susceptible to HIV-1 infection due to hormonal changes, vaginal microbial ecology and physiology, and a higher prevalence of sexually transmitted diseases (Sagar *et al*., 2004; Lavreys *et al*., 2004). People who have been infected with one strain of HIV can still be infected later on in their lives by other, more virulent strains.

Infection is unlikely in a single encounter. High rates of infection have been linked to a pattern of overlapping long-term romantic relationships. This allows the virus to quickly spread to multiple partners who in turn infect their partners. A pattern of serial monogamy or occasional casual encounters is associated with lower rates of infection (Epstein, 2007). HIV spreads readily through heterosexual sex in Africa, but less so elsewhere. One possibility being researched is that schistosomiasis, which affects up to 50 per cent of women in parts of Africa, damages the lining of the vagina (Chenine *et al.,* 2007).

2.3.3 Exposure to blood-borne pathogens

This transmission route is particularly relevant to intravenous drug users, hemophiliacs and recipients of blood transfusions and blood products. Sharing and reusing syringes contaminated with HIV-infected blood represents a major risk for

infection with HIV. Needle sharing is the cause of one third of all new HIV-infections in North America, China, and Eastern Europe. The risk of being infected with HIV from a single prick with a needle that has been used on an HIV-infected person is thought to be about 1 in 150. Post-exposure prophylaxis with anti-HIV drugs can further reduce this risk (Fan, 2005). This route can also affect people who give and receive tattoos and piercings. Universal precautions are frequently not followed in both sub-Saharan Africa and much of Asia because of both a shortage of supplies and inadequate training. The WHO estimates that approximately 2.5% of all HIV infections in sub-Saharan Africa are transmitted through unsafe healthcare injections (WHO, UNAIDS, 2003). Because of this, the United Nations General Assembly has urged the nations of the world to implement precautions to prevent HIV transmission by health workers UNAIDS.

The risk of transmitting HIV to blood transfusion recipients is extremely low in developed countries where improved donor selection and HIV screening is performed. However, according to the WHO, the overwhelming majority of the world's population does not have access to safe blood and between 5% and 10% of the world's HIV infections come from transfusion of infected blood and blood products (WHO, 2001).

2.3.4 Perinatal transmission

The transmission of the virus from the mother to the child can occur in utero during the last weeks of pregnancy and at childbirth. In the absence of treatment, the transmission rate between a mother and her child during pregnancy, labor and delivery is 25%. However, when the mother takes antiretroviral therapy and gives birth by caesarean section, the rate of transmission is just 1% (Coovadia, 2004). The risk of infection is influenced by the viral load of the mother at birth, with the higher the viral

load, the higher the risk. Breastfeeding also increases the risk of transmission by about 4 % (Coovadia and Bland, 2007).

2.4 Pathophysiology

The pathophysiology of AIDS is complex, as is the case with all syndromes (Guss, 1994). Ultimately, HIV causes AIDS by depleting $CD4^+$ T helper lymphocytes. This weakens the immune system and allows opportunistic infections. T lymphocytes are essential to the immune response and without them; the body cannot fight infections or kill cancerous cells. The mechanism of $CD4^+$ T cell depletion differs in the acute and chronic phases (Hel *et al*., 2006). During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for $CD4^+$ T cell depletion, although apoptosis may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in $CD4^+$ T cell numbers.

Although the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^+$ T cell loss occurs during the first weeks of infection, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body (Mehandru *et al*., 2004). The reason for the preferential loss of mucosal $CD4^+$ T cells is that a majority of mucosal $CD4^+$ T cells express the CCR5 coreceptor, whereas a small fraction of $CD4^+$ T cells in the bloodstream do so (Brenchley *et al*., 2004). HIV seeks out and destroys CCR5 expressing $CD4^+$ cells during acute infection. A vigorous immune response eventually controls the infection and initiates the clinically latent phase. However, $CD4^+$ T cells in

mucosal tissues remain depleted throughout the infection, although enough remain to initially ward off life-threatening infections.

Continuous HIV replication results in a state of generalized immune activation persisting throughout the chronic phase (Appay and Sauce, 2008). Immune activation, which is reflected by the increased activation state of immune cells and release of proinflammatory cytokines, results from the activity of several HIV gene products and the immune response to ongoing HIV replication. Another cause is the breakdown of the immune surveillance system of the mucosal barrier caused by the depletion of mucosal $CD4^+$ T cells during the acute phase of disease (Brenchley *et al*., 2006). This results in the systemic exposure of the immune system to microbial components of the gut's normal flora, which in a healthy person is kept in check by the mucosal immune system. The activation and proliferation of T cells that results from immune activation provides fresh targets for HIV infection. However, direct killing by HIV alone cannot account for the observed depletion of $CD4^+$ T cells since only 0.01-0.10% of $CD4^+$ T cells in the blood are infected. A major cause of $CD4^+$ T cell loss appears to result from their heightened susceptibility to apoptosis when the immune system remains activated. Although new T cells are continuously produced by the thymus to replace the ones lost, the regenerative capacity of the thymus is slowly destroyed by direct infection of its thymocytes by HIV. Eventually, the minimal number of $CD4^+$ T cells necessary to maintain a sufficient immune response is lost, leading to AIDS.

2.4.1 Target cells affected by HIV during infection

The virus, entering through whichever route, acts primarily on the following cells: (Harsh, 2005)

- Lymphoreticular system:
    - CD_4+ T-Helper cells
    - CD_4+ Macrophages
    - CD_4+ Monocytes
    - B-lymphocytes
- Certain endothelial cells
- Central nervous system:
    - Microglia of the nervous system
    - Astrocytes
    - Oligodendrocytes
    - Neurones - indirectly by the action of cytokines and the gp-120

2.4.2 The effect of HIV on target cells

The virus has cytopathic effects but how it does it is still not quite clear. It can remain inactive in these cells for long periods, though. This effect is hypothesized to be due to the CD_4-gp120 interaction (Harsh, 2005).

- The most prominent effect of the HIV virus is its T-helper cell suppression and lysis. The cell is simply killed off or deranged to the point of being function-less (they do not respond to foreign antigens). The infected B-cells cannot produce enough antibodies either. Thus the immune system collapses leading to the familiar AIDS complications, like infections and neoplasms.

- Infection of the cells of the Central Nervous System (CNS) cause acute aseptic meningitis, subacute encephalitis, vacuolar myelopathy and peripheral neuropathy. Later it leads to even AIDS dementia complex.
- The CD_4-gp120 interaction (vide supra) is also permissive to other viruses like Cytomegalovirus, Hepatitis virus, Herpes simplex virus, etc. These viruses lead to further cell damage i.e. cytopathy.

2.5 Structure and Genome

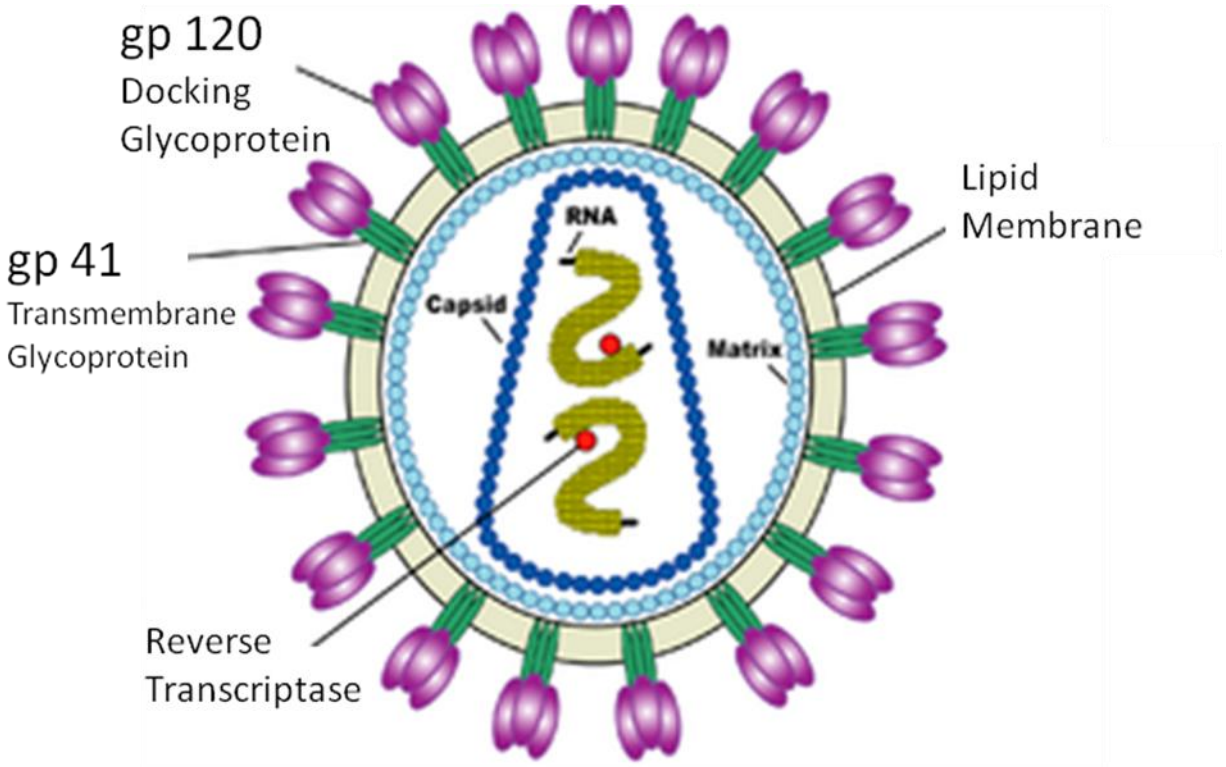

Figure 2.3: Structure of HIV showing viral genes, proteins, nucliec acid and viral enzymes (Wikipedia, 2008).

HIV is different in structure from other retroviruses. It is roughly spherical (McGovern *et al*., 2002) with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus (Fisher et al). It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7 and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle (HSC, 2008). This is, in turn, surrounded by the viral envelope which is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle (HSC, 2008). This protein, known as Env, consists of a cap made of three molecules called glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope (Chan *et al*., 1997). This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle (Chan *et al*., 1997). Both these surface proteins, especially gp120, have been considered as targets of future treatments or vaccines against HIV (NIH, 1998).

The RNA genome consists of at least 7 structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, INS) and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and *tev*) encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles (HSC, 2008). For example,

env codes for a protein called gp160 that is broken down by a viral enzyme to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease (HSC, 2008). The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cell protein which deaminates DNA: RNA hybrids and/or interferes with the Pol protein). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) downregulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules (Garcia *et al*., 1991; Schwartz *et al*., 1996; Stumptner-Cuvelette *et al*., 2001). Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells. The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by Gag and Rev Proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol (HSC, 2008).

2.5.1 Tropism

The term viral tropism refers to which cell types HIV infects. HIV can infect a variety of immune cells such as $CD4^+$ T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and $CD4^+$ T cells is mediated through interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine coreceptors (Chan *et al*., 1997).

Macrophage (M-tropic) strains of HIV-1, or non-syncitia-inducing strains (NSI) use the β-chemokine receptor CCR5 for entry and are thus able to replicate in macrophages and $CD4^+$ T cells (Coakley *et al*.,2005). This CCR5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when $CD4^+$ cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system. In tonsils and adenoids of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amounts of virus.

T-tropic isolates, or syncitia-inducing (SI) strains replicate in primary $CD4^+$ T cells as well as in macrophages and use the α-chemokine receptor, CXCR4, for entry (Coakley *et al*., 2005; Deng *et al*., 1996; Feng *et al*., 1996). Dual-tropic HIV-1 strains are thought to be transitional strains of the HIV-1 virus and thus are able to use both CCR5 and CXCR4 as co-receptors for viral entry.

The α-chemokine SDF-1, a ligand for CXCR4, suppresses replication of T-tropic HIV-1 isolates. It does this by down-regulating the expression of CXCR4 on the surface of these cells. HIV that use only the CCR5 receptor are termed R5, those that only use CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection (Coakley *et al*., 2005) and HIV can also infect a subtype of myeloid dendritic cells, (Knight *et al*., 1990) which probably constitute a reservoir that maintains infection when $CD4^+$ T cell numbers have declined to extremely low levels.

Some people are resistant to certain strains of HIV (Tang *et al*., 2003). One example of how this occurs is people with the CCR5-Δ32 mutation; these people are resistant to infection with R5 virus as the mutation stops HIV from binding to this coreceptor, reducing its ability to infect target cells.

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid which is passed from a male to his sexual partner. The virions can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway (Zhu *et al*., 1993; van't Wout *et al*., 1994; Zhu *et al*., 1996). How this selective process works is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXCR4 on their surface (Muciaccia *et al*., 2005) and that genital epithelial cells preferentially sequester X4 virus (Berlier *et al*., 2005). In patients infected with subtype B HIV-1, there is often a co-receptor switch in late-stage disease and T-tropic variants

appear that can infect a variety of T cells through CXCR4 (Clevestig *et al*., 2005). These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse, and opportunistic infections that mark the advent of AIDS (Moore, 1997). Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS. A number of studies with subtype B-infected individuals have determined that between 40 and 50% of AIDS patients can harbour viruses of the SI, and presumably the X4, phenotype (Karlsson *et al*., 1994; Koot *et al*., 1996).

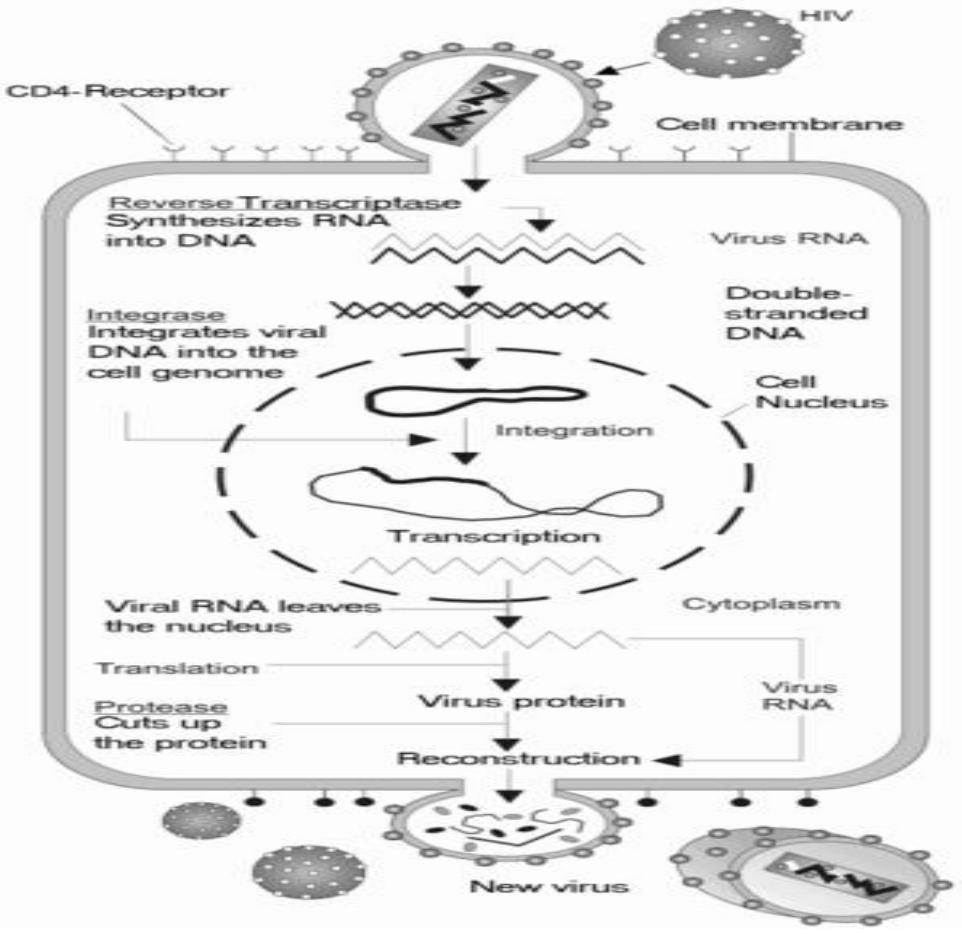

Figure 2.4: Replication cycle of HIV showing the various stages of the virus life cycle. Source reference: AIDS - Wikipedia, the free encyclopedia/replication.mht

2.6 The replicative cycle

2.6.1 Entry to the cell

HIV enters macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface (Chan and Kim, 1998; Wyatt and Sodroski, 1998) gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1 (Arthos *et al*., 2008). The gp160 spike contains binding domains for both CD4 and chemokine receptors (Chan and Kim, 1998; Wyatt and Sodroski, 1998). The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor (Chan and Kim, 1998; Wyatt and Sodroski., 1998). This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane (Chan and Kim, 1998; Wyatt and Sodroski, 1998). Repeat sequences in gp41, HR1 and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close

together, allowing fusion of the membranes and subsequent entry of the viral capsid (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

Once HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease and protease, are injected into the cell (Chan and Kim, 1998). During the microtubule based transport to the nucleus, the viral single strand RNA genome is transcribed into double strand DNA, which is then integrated into a host chromosome.

HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used (Pope and Haase, 2003). DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T cells once the virus has been captured in the mucosa by DCs (Pope and Haase, 2003).

2.6.2 Replication and transcription

Once the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+)RNA from the attached viral proteins and copies it into a complementary DNA (Zheng *et al*., 2005). This process of reverse transcription is extremely error-prone and it is during this step that mutations may occur. Such mutations may cause drug resistance. The reverse transcriptase then makes a complementary DNA strand to form a double-stranded viral DNA intermediate (vDNA). This vDNA is then transported into the cell nucleus. The integration of the viral DNA

into the host cell's genome is carried out by another viral enzyme called *integrase* (Zheng *et al*., 2005).

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection (Zheng *et al*., 2005). To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T cells become activated **(**Hiscott *et al*., 2001). This means that those cells most likely to be killed by HIV are those currently fighting infection.

In this replication process, the integrated provirus is copied to mRNA which is then spliced into smaller pieces. These small pieces produce the regulatory proteins Tat (which encourages new virus production) and Rev. As Rev accumulates it gradually starts to inhibit mRNA splicing. At this stage, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to the Gag protein and is packaged into new virus particles.

HIV-1 and HIV-2 appear to package their RNA differently; HIV-1 will bind to any appropriate RNA whereas HIV-2 will preferentially bind to the mRNA which was used to create the Gag protein itself. This may mean that HIV-1 is better able to mutate (HIV-1 infection progresses to AIDS faster than HIV-2 infection and is responsible for the majority of global infections).

2.6.3 Assembly and release

The final step of the viral cycle, assembly of new HIV-1 virons, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by protease and processed into the two HIV envelope glycoproteins gp41 and gp120. These are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell. Maturation either occurs in the forming bud or in the immature virion after it buds from the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins and enzymes. The various structural components then assemble to produce a mature HIV virion (Gelderblom, 1997). This cleavage step can be inhibited by protease inhibitors. The mature virus is then able to infect another cell.

2.7 Genetic variability

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of 10^9 to 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase (Robertson *et al*., 1995). This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day (Robertson *et al*., 1995). This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes (Robertson *et al*., 1995).

Three groups of HIV-1 have been identified on the basis of differences in *env*: M, N, and O **(**Thomson *et al*., 2002). Group M is the most prevalent and is subdivided into eight subtypes (or clades), based on the whole genome, which are geographically distinct (Carr *et al*., 1998). The most prevalent are subtypes B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia); these subtypes form branches in the phylogenetic tree representing the lineage of the M group of HIV-1. Coinfection with distinct subtypes gives rise to circulating recombinant forms (CRFs). In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2 percent of infections worldwide were of

subtype C, 26.7 percent were of subtype A/CRF02_AG, 12.3 percent were of subtype B, 5.3 percent were of subtype D, 3.2 percent were of CRF_AE, and the remaining 5.3 percent were composed of other subtypes and CRFs (Osmanov *et al*., 2002). Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes (Perrin *et al*., 2003). The genetic sequence of HIV-2 is only partially homologous to HIV-1 and more closely resembles that of SIV than HIV-1.

2.8 HIV plasma viral load.

Viral load is a measure of the amount of HIV copies in a milliliter (copies/mL) of blood. Along with CD4 counts and clinical response HIV viral load is a strong indicator to monitor the success or failure of ART. Viral load measurements are used to determine the risk of disease progression, to decide when to initiate ART, to monitor response to treatment, and to detect viral breakthrough as a marker of regimen failure. Viral load response is also used as a surrogate marker for efficacy in ART drug trials and in clinical practice. Hence, determination of viral load is a significantly important part of monitoring the therapy of HIV-infected and AIDS patients.

2.8.1 Meaning of plasma viral load test results.

HIV viral load tests are reported as the number of HIV copies in a milliliter (copies/mL) of blood. If the viral load measurement is high, it indicates that HIV is reproducing and that the disease will likely progress faster than if the viral load is low. During treatment and monitoring, a high viral load can be anywhere from 5,000 to 10,000 copies/mL. Initial, untreated, and uncontrolled HIV viral loads can range as high as one million or more copies/mL. A low viral load is usually between 40 to 500

copies/mL, depending on the type of test used. This result indicates that HIV is not actively reproducing and that the risk of disease progression is low.

A viral load result that reads “undetectable” does not mean that you are cured. It may mean that either the HIV RNA is not present in your blood at the time of testing or that the level of HIV RNA is below the threshold needed for detection. Even though HIV may be undetectable in the blood, it persists in cells and tissues throughout the body as “HIV provirus.” HIV provirus refers to virus that has moved into cells and into the nucleus, where it has become integrated with the DNA of the host cell. This is also call “HIV proviral DNA.”

Change in viral load is also a very important measurement. A rising count indicates either that the infection is getting worse or that you have developed resistance to the drugs that are being used for therapy, while a falling count indicates improvement and suppression of the HIV infection.

2.9 Treatment.

There is currently no vaccine or cure for HIV or AIDS (Robb, 2008)). The only known method of prevention is avoiding exposure to the virus. However, a course of antiretroviral treatment administered immediately after exposure, referred to as post-exposure prophylaxis, is believed to reduce the risk of infection if begun as quickly as possible (Fan, 2005). Current treatment for HIV infection consists of highly active antiretroviral therapy, or HAART (DHHS, 2005). This has been highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based HAART initially became available (Palella *et al.*, 1998). Current HAART options are combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes," of antiretroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI). New classes of drugs such as Entry Inhibitors provide treatment options for patients who are infected with viruses already resistant to common therapies, although they are not widely available and not typically accessible in resource-limited settings. Because AIDS progression in children is more rapid and less predictable than in adults, particularly in young infants, more aggressive treatment is recommended for children than adults (DHHS, 2005). In developed countries where HAART is available, doctors assess their patients thoroughly: measuring the viral load, how fast CD4 declines, and patient readiness. They then decide when to recommend starting treatment.

HAART neither cures the patient nor does it uniformly remove all symptoms; high levels of HIV-1, often HAART resistant, return if treatment is stopped (Martinez-Picado *et al*., 2000; Dybul *et al*., 2002). Moreover, it would take more than a lifetime for HIV infection to be cleared using HAART (Blankson *et al*., 2002). Despite this, many HIV-infected individuals have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world (Palella *et al*., 1998; Wood et al., 2003; Chene *et al*., 2003). One study suggests the average life expectancy of an HIV infected individual is 32 years from the time of infection if treatment is started when the CD4 count is 350/μL (Schackman *et al*., 2006). In the absence of HAART, progression from HIV infection to AIDS has been observed to occur at a median of between nine to ten years and the median survival time after developing AIDS is only 9.2 months (Morgan *et al*., 2002). However, non-adherence with antiretroviral therapy is the major reason most individuals fail to benefit from HAART (Becker *et al*., 2002). The reasons for non-adherence with HAART are varied and overlapping. Major psychosocial issues, such as poor access to medical care, inadequate social supports, psychiatric disease and drug abuse contribute to non-adherence. The complexity of these HAART regimens, whether due to pill number, dosing frequency, meal restrictions or other issues along with side effects that create intentional non-adherence also contribute to this problem (Nieuwkerk *et al*., 2001; Kleberger *et al*., 2001; Heath *et al*., 2002). The side effects include lipodystrophy, dyslipidemia, insulin resistance, an increase in cardiovascular risks and birth defects (Montessori *et al*., 2004; Saitoh *et al*., 2005).

The timing for starting HIV treatment is still debated. There is no question that treatment should be started before the patient's CD4 count falls below 200, and most national guidelines say to start treatment once the CD4 count falls below 350; however, these two school of thoughts notwithstanding some evidence from cohort studies that treatment should be started before the CD4 count falls below 350 (Wang *et al*., 2004; Wood *et al*., 2003). In those countries where CD4 counts are not available, patients with WHO stage III or IV disease (WHO, 2006) should be offered treatment.

Anti-retroviral drugs are expensive, and the majority of the world's infected individuals do not have access to medications and treatments for HIV and AIDS (Ferrantelli *et al*., 2004). Research to improve current treatments includes decreasing side effects of current drugs, further simplifying drug regimens to improve adherence, and determining the best sequence of regimens to manage drug resistance. Unfortunately, only a vaccine is thought to be able to halt the pandemic. This is because a vaccine would cost less, thus being affordable for developing countries, and would not require daily treatment (Ferrantelli *et al*., 2004). However, after over 20 years of research, HIV-1 remains a difficult target for a vaccine (Ferrantelli *et al*., 2004).

2.9.1 Alternative medicine

The term alternative medicine, as used in the modern western world, encompasses any healing practice "that does not fall within the realm of conventional medicine (Bratman and Steven, 1997)." Commonly cited examples include naturopathy, chiropractic, herbalism, traditional Chinese medicine, Ayurveda, meditation, yoga, biofeedback, hypnosis, homeopathy, acupuncture, and diet-based therapies, in addition

to a range of other practices. Various forms of alternative medicine have been used to treat symptoms or alter the course of the disease (Power *et al*., 2002). Acupuncture has been used to alleviate some symptoms, such peripheral neuropathy, but cannot cure the HIV infection (Nicholas *et al*., 2007).

Herbal medicines are defined as products derived from plants or parts of plants used for the treatment of pathogen causing infectious diseases such as HIV/AIDS as well as non communicable disease conditions. Some HIV-infected people use herbs for potential cure or symptom treatment due to the fact that they are efficacious, readily available, and more inexpensive (Yolan *et al*., 2007; Kristen, 2007). Even though medicinal plants are popularly assumed to be safe and natural alternative to conventional medications, traditional medicines especially those used for HIV/AIDS treatment are not well researched, and are poorly regulated. Several randomized clinical trials testing the effect of herbal medicines have shown that there is no evidence that these herbs have any effect on the progression of the disease, but may instead produce serious side-effects.

Although there is a lot of interest in herbal medicine and use, unfortunately there is very little data that shows any real effect in relation to HIV/AIDS in Ghana. However several studies have demonstrated the inhibitory properties of a variety of crude plant extracts, as well as chemically characterized phytomolecules against different stages of the life cycle of HIV. Some of these studies focused on plant parts used traditionally in specific geographic locales in the treatment of various forms of infectious diseases. Interestingly, a few plant derived compounds such as papavarine glycyrrhizin and trichosanthin were seen to have promise and have been evaluated in AIDS patients.

In a study to clinically assess the efficacy of South African traditional medicine on viral load and CD4 counts, (Tshibangu et al., 2004) described an improvement in the immune system and general well-being of patients, due to increases in CD4+ T cell and decrease in viral load, when these markers were monitored for 12 months in HIV/AIDS participants. These developments show that useful anti-HIV agents could be obtained from plants sources (Vlietinck et al., 1998; De Clercq, 2000; Kong et al., 2003), as well as proves that herbal medicines might have the potential to alleviate symptoms, reduce viral load, and increase CD4+ cells for HIV-infected individuals and AIDS patients (Burack, 1996; Durant, 1998; Kang, 1999; Liu, 2000; Lu, 1993; Zheng, 1999). On the other hand, there is an increasing number of reports in the medical literature about liver toxicity, kidney failure and other adverse events from some herbal products (Ishizaki, 1996; Melchart, 1999), as well as possible herb-drug interactions (Izzo, 2001).

This study is aimed at determining the response of HIV/AIDS patient to some herbal products using virological (viral load) changes in correlation with $CD4^+$ T cells count success or failure. This will help to establish whether to recommend them as viable alternative therapy for the treatment of HIV/AIDS.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Selection of herbalist, herbal centres and their role

There are many traditional herbal practitioners in Ghana who treats various kinds of diseases including HIV/AIDS. A number of them were contacted out of which six who have had validated their herbal products were selected. The six were the following herbal centres:

1. M and J Health Management Consult, Mampong.
2. Obeng Memorial Herbal Clinic, Pankrono, Kumasi.
3. Amansan Boafo Herbal Centre, Kronom New Site, Kumasi.
4. Yehowa Behwe Herbal Centre, Atwima-Brofoyedu, Kumasi.
5. Tawheed Naturopathic Clinic, Boadi, Kumasi.
6. Akobalm Herbal Enterprise, Sepetimpom, Kumasi.

A seminar was organized where the herbalists were briefed on the objectives of the study, its importance and their role. Each herbalist was to present three HIV/AIDS patients who will be willing to take their herbal products to be administered by the herbalist themselves. Moreover they were made aware that they will present their patients for blood sample collection at the end of every month throughout the study period. The six herbal centers were given codes (C001-C006) as M and J Health Management Consult -C001; Obeng Memorial Herbal Clinic - C002; Amansan Boafo Herbal Centre - C003, Yehowa Behwe Herbal Centre - C004; Tawheed Naturopathic Clinic - C005 and; Akobalm Herbal Enterprise - C006. M and J Health Management

Consult, Obeng Memorial Herbal Clinic and Amansan Boafo Herbal Centre were the only centres that participated in the study because they were able to meet the requirements of providing their own patients. Each herbalist was given questionnaire (appendix 1) requiring information on their herbal centre as shown in (Table 3.1) and herbal products profile shown in (Table 3.2) to complete. All the herbalists signed an informed consent form committing them to the study.

Table 3.1: The herbal centres profile showing their address, years of practice, diseases treated at the centre, etc.

Centre profile	**Centre 1**	**Centre 2**	**Centre 3**
Name	M and Jay Health Management Consult	Obeng Memorial Herbal clinic	Amansa Boafo
Type of herbal centre	Organization	Individually owned	Individually owned
Location address	Mampong	Kumasi	Kumasi
Years of practice	8 years	12 years	14 years
Diseases treated at the centre	HIV/AIDS, diabetic wounds, buruli ulcer, hepatitis, liver cirrhosis	All diseases	HIV/AIDS and all other diseases
When HIV/AIDS treatment begun at the centre	1999	1992	1994
Other diseases treated by the HIV/AIDS product	None	Broad spectrum of infections and cancers	Asthma, whites, chronic diarrhoea, breast cancer, eye disease

Table 3.1 continued

Number of HIV patients treated so far	720	26	100 and over
Criteria for selection of patients for HIV treatment	If one can guarantee accessibility to nutritional support and to control secondary infections.	When CD4 levels are stable without drugs for 6 months	Symptoms of HIV/AIDS: Weight loss, persistent fever etc.
How response to treatment is monitored	CD4 cell count, LFT & RFT PRN positive change in appetite if clinically symptomatic	Through laboratory results or when opportunistic disease declines	When symptoms disappear
Duration of the treatment process	6 months, but WHO category 4 as long as possible based on indicators	6 to 12 months	Between 6 and 8 months

Table 3.2: The herbal products profile showing the materials used in preparation, shelf- life, dosage, treatment schedule etc.

Drug Profile	**Centre 1**	**Centre 2**	**Centre 3**
Name of herbal drug	MJ GOLDONI	Misparon OA, Unity mixtures	Amansan Boafo
How potency of drug was realized	Changes in CD4 counts morphological & appetite changes in clinically symptomatic clients	Through in vivo &in vitro test and clinical evidence	After trying it on some HIV positive patients recommended by doctors
Nature	Liquid	Liquid	Liquid
Method of preparation	Water extraction	Essence oil extraction	Water extraction
Materials used in preparation	*Plants part specific	Plants roots and stem bark	Nyamedua nhini, mahogany, etc which part
Maintenance	Stabilized with sodium benzoate, keep in cool place		Mixed sodium benzyl
Shelf-life	One and half years	Three years	Three years

Table 3.2 continued

Administration	Orally	Orally	Orally
Treatment schedule	Three times daily	Three times daily	Three times daily
Dosage	Dependent on WHO category before treatment	45mls	One tablespoonful
Known side effects	Suppression of appetite if misapplied, transient acute weakness, hepatotoxicity if misapplied in WHO category 4 clients	None	None

3.2 Selection of Patients.

The patients presented by the herbalists were briefed during a seminar on the importance of the study, their role and how the study would be conducted. Those who submitted laboratory results to proof that they are HIV/AIDS positive and are therapy naive were selected to participate in the study. In all, six candidates consisting of three males and three females were selected. They were administered questionnaire regarding information on their background. Patients C001/P01, C001/P02, C001/P03 were recruited by M and J Health Management Consult, patients C002/P01 and C002/P02 were recruited by Obeng Memorial Herbal Clinic and patient C003/P01 was recruited by Amansan Boafo Herbal Centre. The patients committed themselves to the study by

signing an informed consent form (appendix II). The HIV antibody status of the patients was confirmed using Determine HIV 1/2 and OraQuick HIV 1/2 antibody tests.

Table 3.3 below gives information on the identity of patients, their background as well as the centre that recruited them.

Table 3.3 The patients profile showing sex, age, marital status, religion, clinical condition etc.

PATIENT ID	**CENTER CODE**	**SEX**	**AGE**	**MARITAL STATUS**	**EDUCATION**	**RELIGION**
C001/P01	C001	F	31	Single	Primary	Christianity
C001/P02	C001	F	44	Divorced	Primary	Christianity
C001/P03	C001	M	45	Divorced	Secondary	Christianity
C002/P01	C002	M	40	Married	Primary	Christianity
C001/902	C002	F	38	Married	None	Christianity
C003/P01	C003	M	34	Single	Primary	Islam

Table 3.3 continued

PATIENT ID	ECONOMIC STATUS	HABITAT	HIV STATUS	CLINICAL CONDITIONS
C001/P01	Low income	Urban	Positive	-
C001/P02	Low income	Urban	Positive	HIV wasting syndrome stage 3
C001/P03	Low income	Semi-Urban	Positive	Chronic diarrhoea stage 3
C002/P01	Low income	Semi-Urban	Positive	Chronic diarrhoea stage 3
C002/P02	Low income	Semi-Urban	Positive	-
C003/P01	Low income	Rural	Positive	Oral candidiasis stage 3

3.3 How the study was conducted.

Patient's physical and clinical conditions were examined by a qualified medical practitioner before the study began and after every two months.

Blood specimen collection began in June when patients were not on herbal therapy. Subsequent collections were made at the beginning each month from July to December as the patients were still on their herbal therapy. The blood specimen were processed and used for the tests below.

3.4 Laboratory tests.

The evaluation of the herbal products was carried out by conducting the following laboratory tests before and after the initiation of the herbal therapy:

1. HIV Antibody tests.
2. CD4 cell count.
3. Heamatology.
4. Biochemistry.
5. Plasma Viral Load.

3.4.1 Specimen collection, processing and storage.

Venous blood specimen was collected into ethylenediaminetetraacetic acid (EDTA) tubes and vacutainer tubes labeled with patient's identification. Part of the blood collected into the ethylenediaminetetraacetic acid (EDTA) tubes was used for the haematology and CD4 cell count measurements within 3 hours of collection. The remaining blood specimen in the EDTA tubes and the vacutainer tubes were centrifuged within 6 hours of collection into plasma and serum respectively. The plasma was then kept in two aliquots of five hundred microlitres (500ul) with the serum being kept in four aliquots of five hundred microlitres (500ul) after which all were frozen at −70°C. The serum was used for the detection of the cytokine levels, HIV antibody tests and the chemistry whiles the plasma was used for the measurement of the plasma viral load analysis. The above was done once every month from June to December 2008.

3.4.2 HIV Antibody Tests.

This test was intended as an aid to detect antibodies to HIV-1/HIV-2 from the infected individuals. In our study the HIV antibody status was confirmed using Abbott Determine HIV-1/2 (ABBOT, Minato-ku, Tokyo, Japan) and OraQuick HIV-1/2 (Orasure Technologies, Bethlehem, PA, USA). The test was repeated at the beginning of every month to find out if there could be a change in the HIV antibody status of patients as claimed by some of the herbalist.

3.4.2.1 Abbott Determine HIV-1/2 is an *in Vitro*, visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, whole blood or plasma. In the experiment fifty microlitres (50µl) of serum was added to the sample pad and the results were read twenty (20) minutes later. The results were read as POSITIVE when red bars appeared in both the control window (labeled "C") and the patient window (labeled "T") of the strip or NEGATIVE when one red bar appeared in the control window (labeled "C"), and no red bar appeared in the patient window of the strip (labeled "T").

3.4.2.2 OraQuick HIV-1/2 is a visually read qualitative immunochromatographic test intended for the detection of antibodies to HIV-1 and HIV-2 in human serum, oral fluid, whole blood or plasma. In the test five microlitres (5µl) of serum was transferred with a micropipette into the vial of developer solution. The loop was immersed into the developer and stirred gently to mix. The pad end of the test device was inserted all the way down into the vial and the results were read twenty (20) minutes later.

The results were read as NON-REACTIVE when a single line appeared on the test strip in the area adjacent to the triangle labeled “C” or REACTIVE when two lines appeared on the test strip, adjacent to the “T” and “C” triangles respectively.

For all our samples tested the results were POSITIVE (REACTIVE) throughout the study.

3.4.3 Heamatology analysis.

The heamatologic parameters heamoglobin, hematocrit, white blood cells, platelets, and differential count were analysed using the Mindray BC-3000 Auto Heamatology Analyser (MINDRAY, Nanshan, Shenzhen, China). (Fig 3.1A and B).

One millilitre (1ml) of the blood specimen collected into anticoagulant (EDTA) tube was gently turned up and down to ensure a uniform mixture. The aspirator on the Mindray BC-3000 Auto Heamatology Analyser was dipped into the blood specimen in the anticoagulant tube (Fig 3.1B). The results for the heamatologic parameters were printed out of the equipment after about a minute (Fig 3.1A).

Figure 3.1A: MINDRAY BC-3000

Figure 3.1B: Blood fed to the aspirator.

3.4.4 CD4+ T cell count.

The enumeration of CD4 lymphocyte numbers was carried out on the blood collected into EDTA-containing tubes by SP flow cytometry (Trucount) on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). (Fig 3.2).

Twenty microliter (20µl) of the monoclonal antibodies was pipetted into the Trucount tube. Subsequently, fifty microlitres (50µl) of well-mixed whole blood was pipetted, capped, vortexed and incubated in the dark at room temperature for 15 min. Then four hundred and fifty microlitre (450µl) of FACS Lysing solution was added and incubated for a further 15 min, followed by an analysis on the flow cytometer. The CD4 T-cell count was computed by the Multiset software (BD Biosciences, San Jose, CA, USA) using the formula: CD4 T-cell count=sample CD4 events/bead events × (bead count/50). The results of each patients sample were then recorded.

Figure 3.2 FACS Calibur Flow Cytometer

3.4.5 Chemistry of patients.

Chemistry of the patients was read on BT 3000 PLUS (Biotecnica Instruments, Via Licenza, Rome, Italy) an automated analyser (Fig 3.3).

Fifty microlitres (50µl) of serum was put into a sample cup labelled according to the samples. The samples identification was entered alongside the liver and renal function tests to be run on the chameleon. These were uploaded onto the BT 3000 PLUS after which the samples were loaded onto its sample plates. Command run was then issued and the results read 30 minutes later.

Figure 3.3: BT 3000 PLUS

3.4.6 Viral load (HIV RNA levels) measurement

Plasma HIV RNA (viral load) testing quantifies the HIV viral burden in the plasma.

HIV RNA levels were measured in plasma prepared from blood that had been collected into EDTA-containing tubes and stored at -70°C using the COBAS AMPLICOR version 1.5 (Roche Molecular Systems, Branchburg, NJ, USA). (fig 3.4)

Principles of the procedure

The Cobas Amplicor v 1.5 is based on five major processes:

1. Sample processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA).
3. PCR amplification of target cDNA using HIV-1 specific complementary primers.
4. Hybridization of the amplified products to oligonucleotide probes specific to the target(s).
5. Detection of the probe-bound amplified products by colorimetric determination.

The test consists of independent steps for RNA isolation, reverse transcription and PCR (RT-PCR) amplification, and detection using a colorimetric readout. Viral RNA was released from the virions with guanidine isothiocyanate, and the ribonucleic acid (RNA) formed from the relatively impure lysate was precipitated with isopropanol. The COBAS Amplicor analyzer was used for the reverse transcription, Polymerase Chain Reaction (PCR) amplification, hybridation and detection to produce the results.

Figure 3.4: The COBAS Amplicor analyser.

CHAPTER FOUR

4.0 RESULTS

4.1 Constraints of the study.

A total of six individuals qualified for inclusion in the study. Of the six patients, centre C001 provided three; centre C002 provided two while centre C003 provided only one. They consisted of equal number of males and females. Two of them were excluded because patient C001/P01 died two months into the study and patient C002/P01 discontinued after three months as a result of her relocation to very far place.

4.2 Description of herbal centres.

M and J Health Management Consult, Obeng Memorial Herbal Clinic, and Amansan Boafo Herbal Centre were the centres able to provide some patients though not to the required number. Tawheed Naturopathic Clinic, Yehowa Behwe Herbal Centre and Akobalm Herbal Enterprise could not provide any patients.

M and J Health Management Consult (C001) is an organization located at Mampong in the Ashanti region and has been practicing for the past 8 years. The centre administers treatment for HIV/AIDS, diabetes, buruli ulcer etc. MJ GOLDONI is the herbal product they use to treat HIV/AIDS and determines its response to treatment by considering Liver Function and Renal Function Tests as well as CD4 cell count. The centre had as at the beginning of the study treated a total of 720 HIV/AIDS patients since 1999. It takes this organization 6 months to treat HIV/AIDS.

Obeng Memorial Herbal Clinic (C002) which is located in Pankrono, Kumasi has been practicing for the past 12 years. It is an individual herbal clinic which began the treatment of HIV/AIDS in 1992 and had recorded 26 cases of HIV/AIDS as at the beginning of our study. MISPARON OA UNITY MIXTURE is the drug administered for the treatment of HIV/AIDS and response to treatment is assessed by decline in opportunistic infections. The centre treats other diseases in addition to HIV/AIDS and it takes between 6-12 months for this centre to treat the disease.

Amansan Boafo (C003) is an individual herbal centre practicing at Kronom New Site, Kumasi for the past 14 years. The centre began the treatment of HIV/AIDS in 1994 with the herbal product Amansan Boafo and had recorded more than 100 cases treatment as at May 2008. Amansan Boafo treats breast cancer, eye infections, asthma, chronic diarrhoea and candidiasis alongside HIV. Response to treatment is monitored by decline in the symptoms of the infection. Amansan Boafo takes between 6-8 months to treat HIV/AIDS.

4.3 Description of herbal products.

The following herbal products being evaluated were produced by the three herbal centres considered for the study.

MJ GOLDONI is the herbal product administered by M and J Health Management Consult. It is prepared by extracting plant liquid and administered orally three times daily. This product is known to cause transient acute weakness and loss of

appetite if misapplied. It is maintained by sodium benzoate and has a shelf-life of one and half years.

Obeng Memorial Herbal Clinic administers the herbal product MISPARON OA unity mixture. It is prepared from plant roots and stem barks by essence oil extraction. It is administered orally 45mls three times daily. It has a shelf life of three years with no known side effects.

Amansan Boafo administers a tonic-like herbal product prepared from the roots of Nyamedua and mahogany by extracting the liquid. Its dosage is one table spoonful orally for three times in a day. It is maintained with sodium benzyl and has a shelf life of three years with no known side effects.

4.4 Description of patients.

Out of the six patients who started the study only patients C001/P02, C001/P03, C002/P01 and patient C003/P01 completed successfully. Patient C001/P01 died two months into study and patient C002/P02 was inconsistent and discontinued after three months because she relocated from Kumasi.

Patient C001/P02 recruited by M and J Health Management Consult was a Christian aged 41 years old and educated up to primary school level. She was divorced and lived in an urban community.

Patient C001/P03 who was 45 years and divorced was also recruited by M and J Health Management Consult. He was a Christian with up to secondary education and resident in a semi-urban community in the country.

Patient C002/P01 was 40 years of age and married. He was a primary school leaver, resident in a semi urban area and a Christian recruited by Obeng Memorial Herbal Clinic.

Patient C003/P01, a Muslim, 34 years of age and single resided in a rural area. He was a primary school leaver recruited by Amansan Boafo.

4.5 LABORATORY TESTS

4.5.1 Plasma viral load of patients.

Plasma viral load measured throughout the period in the patients have been shown in Table 4.1.

Patient C001/P02 had baseline plasma viral load of about a million before the herbal therapy was administered. These dropped massively to below 400 copies by the 8^{th} week, where it remained so even at 16^{th} and 24^{th} weeks during the medication.

Similarly patient C001/P03 who was on the same medication as patient C001/P02 started with plasma viral load of about 850,000 copies/ml at baseline before the start of herbal therapy. The level however dropped drastically during the remaining weeks to undetectable levels by the 24^{th} week.

A baseline of 320,000 copies/ml was recorded for patient C002/P01 before the administration of the herbal therapy. The patient however experienced fluctuations in level for the rest of the period. Finally patient C003/P01 also experienced some fluctuations after beginning the study with an initial viral load of 3,130,000 copies/ml.

Table 4.1: Viral load measurements (Copies/ml) before and after herbal product administration throughout the study period.

TREATMENT WEEKS	C001/P02	C001/P03	C002/P01	C003/P01
PRE	9.4×10^5	8.5×10^5	3.2×10^5	3.1×10^6
8	< 400	1.1×10^3	3.1×10^5	1.1×10^6
16	< 400	< 400	2.1×10^5	1.9×10^6
24	< 400	< 400	8.0×10^5	1.2×10^6

4.5.2 CD4+ T cell count of patients.

The CD4 level of patient C001/P02 recorded significant increase from the baseline to the 16th week. The level had however decreased slightly by the 24th week. Patient C001/P03 who was administered the same therapy experienced similar trends as patient C001/P02.

A decrease was recorded throughout the period for patient C002/P01 who was administered Misparon OA unity mixture.

Patient C003/P01 who was administered Amansan Boafo recorded fluctuating CD4 levels during the period.

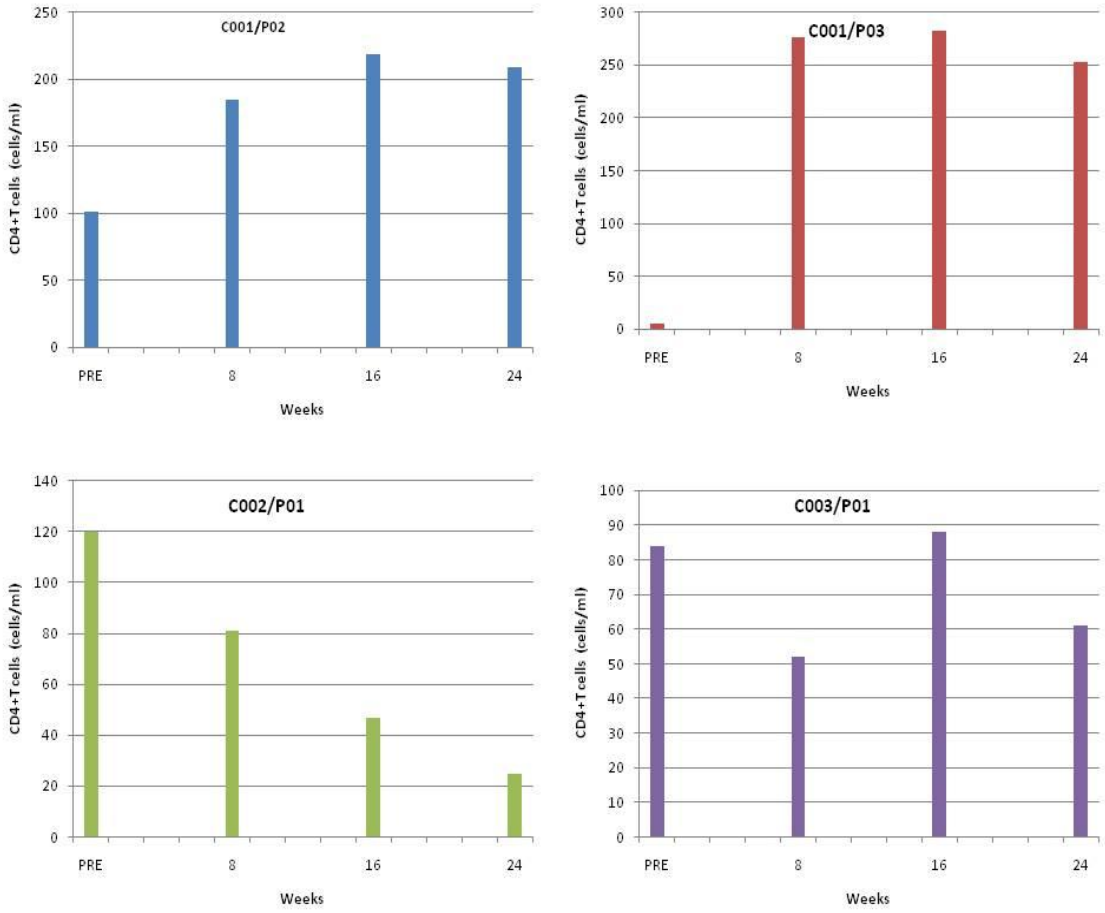

Figure 4.1: Shows the clinical course of patients CD4+ T cell counts before and after herbal product administration throughout the study period.

4.5.4 Haematology profile of patients.

Table 4.2 below shows the haematology profile of the patients throughout the study. Patient C001/P02 and patient C001/P03 recorded normal haemoglobin levels at the end of the study although patient C001/P03 had his baseline haemoglobin slightly decreased from the normal. Patient C002/P01 recorded normal haemoglobin level for the first 8 weeks but decreased by a slight margin for the rest of the period. Patient C003/P01 recorded abnormal haemoglobin level throughout the period.

An abnormal hematocrit level was recorded for patient C001/P02 and patient C001/P03 during the first 8 weeks but turned normal for the rest of the period. Patient C002/P01 recorded normal hematocrit level for up to 8 weeks before becoming abnormal at the end. Patient C003/P01 was however abnormal throughout the period.

The white blood cells (WBC) for all the patients were abnormal except patient C003/P01 who was normal at baseline and remained virtually the same during the study period.

The platelet level of patient C003/P01 was abnormal but the rest had recorded normal levels by the end of the study.

With regards to differential count patient C002/P01 and patient C003/P01 were normal in terms of their granulocyte and lymphocyte levels; however, patient C001/P02 and patient C001/P03 were abnormal for the same parameter. The mid count of patient C002/P01 was normal at the end of the study whiles that of the remaining patients were abnormal.

Table 4.2: Heamatology profile of patients showing the levels of haemoglobin, hematocrit, white blood cells, platelets, etc measured before and after herbal product administration throughout the study period.

HAEMATOLOGY	WEEKS	C001/P02	C001/P03	C002/P01	C003/P01
Hb(g/dl) Ref. range: 11.0-16.0	PRE	10.8	10.3	13.3	8.5
	8	11.5	13.0	13.8	9.6
	16	11.5	13.1	9.3	9.4
	24	12.6	14.7	10	8.4
Hematocrit (%) Ref. range: 37.0-50.0	PRE	29.3	28.8	39.8	22.7
	8	32.6	35.3	40.7	26.7
	16	38.0	41.9	27.0	26.7
	24	38.0	43.2	31.1	22.8
WBC (10^9/L) Ref. range: 4.0-10.0	PRE	3.9	4.0	4.1	5.5
	8	4.6	3.3	4.0	3.0
	16	3.8	3.8	5.9	5.4
	24	3.8	3.5	3.0	5.5
Platelets (10^9/L) Ref. range: 100-300	PRE	208.0	278.0	122.0	153
	8	205.0	254.0	129.0	277
	16	277.0	236.0	392.0	343
	24	277.0	256.0	230	384

Table 4.2 continued

HAEMATOLOGY		WEEKS	C001/ P02	C001/ P03	C002/ P01	C003/ P01
Differential Count (%)	**Gran** Ref. range: 50-70	PRE	30.3	24.1	61.2	44.1
		8	37.9	18.1	45.4	19.9
		16	49.6	22.1	31.9	59.5
		24	30.8	27.0	58.9	67.0
	Lym Ref. range: 20-40	PRE	58.6	68.2	28.1	34.6
		8	54.4	74.9	36.3	72.1
		16	40.7	64.7	48.2	26.1
		24	58.8	65.6	25.7	20.8
	Mid Ref. range: 3-9	PRE	11.1	7.7	10.7	21.3
		8	7.7	7.0	18.3	8.0
		16	9.7	13.2	19.9	14.4
		24	10.4	7.4	15.4	11.6

4.5.5 Chemistry Profile of Patients.

Table 4.2 below shows the chemistry profile of the patients throughout the period. With respect to the liver enzymes the AST recorded for all the patients were normal except that of patient C001/P03 which was abnormal throughout the study period. Moreover the GGT level was abnormal for all the patients except patient C003/P01 who recorded normal level with patient C001/P03 recording very high values. Finally patient C001/P03 recorded a slight deviation of his ALT from the reference range at baseline but however remained normal with the other patients for the rest of the period.

The total bilirubin and Blood Urea Nitrogen (BUN) were normal for all the patients except patient C003/P01 who recorded abnormal BUN throughout the period. The creatinine level of patient C001/P03, patient C002/P01 and patient C003/P01 were abnormal at baseline and at the end but that of patient C001/P02 remained normal throughout the study period.

Table 4.3: Biochemistry profile of patients showing the levels of Gamma-glutamyl transferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), etc measured before and after herbal product administration throughout the study period.

CHEMISTRY	**WEEKS**	**C001/P02**	**C001/P03**	**C002/P01**	**C003/P01**
	PRE	28.0	109.0	30.0	71.0
GGT (U/L)	8	50.0	408.0	31.0	48.0
Ref range: 9-36	16	54.0	236.0	38.0	69.0
	24	51.0	133.0	45.0	35.0
	PRE	0.4	0.4	0.3	0.8
Total Bilirubin (mg/dl)	8	0.1	0.2	0.5	0.3
Ref range: 0.2-1.5	16	0.3	0.5	0.6	0.5
	24	0.4	0.8	0.6	0.8
	PRE	21.0	58.0	19.0	32.0
AST (U/L)	8	26.0	37.0	21.0	18.0
Ref range: 5-40	16	32.0	92.0	33.0	27.0
	24	31.0	49.0	25.0	28.0

Table 4.3 continued

CHEMISTRY	WEEKS	C001/P02	C001/P03	C002/P01	C003/P01
	PRE	5.0	12.0	4.0	4.0
ALT (U/L)	8	8.0	15.0	7.0	6.0
Ref range: 10-40	16	10.0	18.0	11.0	6.0
	24	14.0	26.0	11.0	9.0
	PRE	12.0	11.0	17.0	25.0
BUN (mg/dl)	8	12.0	9.0	16.0	17.0
Ref range: 6-20	16	10.0	16.0	12.0	17.0
	24	13.0	11.0	15.0	23.0
	PRE	0.7	1.2	1.6	1.6
Creatinine (mg/dl)	8	0.7	1.0	1.5	1.1
Ref range: 0.6-1.1	16	0.8	1.4	1.6	1.0
	24	1.0	1.2	1.6	1.2

CHAPTER FIVE

5.0 DISCUSSION

This study was carried out to evaluate the response of HIV/AIDS patients to some herbal products administered to them using virological (viral load) and other parameters such as CD4 cell count, biochemistry and haematology.

5.1 Evaluation of the Biochemistry and haematological profile of patients

Medicinal plants are popularly assumed to be safe and natural alternative to conventional medications, On the other hand, there is an increasing number of reports in the medical literature about liver toxicity, kidney failure and other adverse events from some herbal products (Ishizaki, 1996; Melchart, 1999),which sometimes aggravate the already worsened conditions of those HIV/AIDS patients using them. Haematological and chemistry parameters of the patients were evaluated to find out the possible toxic effects associated with the herbal products administered in our study.

To evaluate the biochemical levels of patients in relationship to toxicity Aspartate aminotransferase (AST), Gamma-glumatyl transferase (GGT), Alanine aminotransferase and total bilirubin were performed to investigate any possible liver disease, while creatinine and blood urea nitrogen were done to investigate possible kidney dysfunction. In terms of haematology the following parameters: platelets, white blood cells, hematocrit, heamoglobin, and differential count were also evaluated.

Our results were interpreted based on comparison with the reference ranges of the parameters evaluated. It was realised that some of the results were abnormal at baseline but became normal at the end while others were normal at baseline but deviated

slightly outside the reference range at the end of the study though did not show any significant difference . The abnormal aspartate aminotransferase (AST), and Gamma-glumatyl transferase (GGT) levels recorded by patient C001/P03 at the end could not be the effect of MJ GOLDONI but probably due to the viral infection since the levels were abnormal before the herbal product was administered. Moreover patient C001/P02 who was administered the same therapy as patient C001/P03 recorded normal aspartate aminotransferase (AST) level throughout the study. Thus the biochemistry and haematology revealed no toxic effect of the herbal products.

5.2 Plasma viral load and CD4+ T cell count.

The goal of HIV medication is to lead to sustained virological suppression and CD4+ T cell count increases which should in time lead to reduced risk of clinical events. However, this can be mostly achieved when there is high level of therapy adherence to medication regimen (Vanhove *et al*., 1966). Patient C001/P02 and C001/P03 attained favorable virological and immunological success to MJ GOLDONI (Tshibangu *et al*., 2004). Thus probably the success may have occurred following the good adherence exhibited by the patients to MJ GOLDONI.

Patient C002/P01 and C003/P01 recorded a seemingly virological and immunological failure as manifested by the increase in their viral load and decreased CD4+ T cell count which could be due to poor adherence to the therapy. This phenomena is in agreement with the observation that non-adherence to antiretroviral therapy is the major reason why most individuals fail to benefit from antiretrovirals (Becker *et al*., 2002).

According to (Bruno et al., 2002) the levels of depression, perception of individual state of mind among others affects adherence. Patient C002/P01 who was administered

MISPARON OA unity mixture had psychological problems and was emotionally depressed due to community denials and stigmatisation. He made persistent claim of committing suicide because of his condition. We think this might be the cause of him not responding favourable to the therapy. Indeed his viral load level initially decreased from the very high baseline value by the eighth and sixteenth week, we therefore think that the herbal product, MISPARON OA unity mixture may be quite potent in controlling HIV/AIDS but the extreme emotional condition of the patient may have antagonize the herbal product's effect. Even though we do not have any evidence to show it we think the improper adherence may have led to the emergence of drug-resistant strains which may have account for the patient's non response to therapy.

Patient C003/P01 was quite interesting because on the first day of his appearance he was too weak to even draw blood from his veins. A month after taking Amansan Boafo, he became stronger and there were no problems associated with the venous blood collection. This had an impact on his viral load level as it was decreased by more than half by the eight week.

When his conditions improved, he started his work again and was not reporting for treatment as required. In fact he reported only when his condition deteriorated, this suggests that his overall condition was improper adherence to therapy just as patient C002/P01.

However the remarkable progress made by C002/P01 and C003/P01 is the best proof of the probable "virus-cidal effect" associated with MISPARON OA UNITY

MIXTURES and AMANSAN BOAFO (Tshibangu *et al*., 2004; Vlietinck *et al*., 1998; De Clercq, 2000; Kong *et al*., 2003).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Following good adherence patient C001/P02 and C001/P03 administered with herbal product MJ GOLDONI experienced virological (viral load) as well as immunological (CD_4+ T cells count) success. Patient C002/P01 and C003/P01 who were administered herbal products MISPARON OA unity mixture and AMANSAN BOAFO respectively experienced both virological and immunological failure due to non-adherence. The achievement of health improvement within six months in terms of viral load and CD_4+ T cells count with MJ GOLDONI indicates that it can be used to control HIV/AIDS. MJ GOLDONI can therefore be said to be a good immune booster and probable "virus-cidal" factor. The response of C002/P01 and C003/P01 to MISPARON OA unity mixture and AMANSAN BOAFO cannot be ascertained since patients were non-adherent. However the remarkable progress made by MISPARON OA UNITY MIXTURES AND AMANSAN BOAFO is the best proof of the probable "virus-cidal effect" associated with these herbal products.

6.2 RECOMMENDATIONS

For our study, MJ GOLDONI produced by M and J Health Management Consult showed favorable response for controlling HIV/AIDS but warrants further research with larger sample size. Due to non-adherence exhibited by the patients who were administered MISPARON OA unity mixture and AMANSAN BOAFO we suggest that these herbal products must be given another chance in a subsequent trials with reliable patients.

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APPENDIX I

SAMPLE OF QUESTIONNAIRE USED TO ACQUIRE PROFILE OF HERBALISTS AND THEIR HERBAL DRUGS

HERBALIST:

1. Name of treatment

centre…….

2. Type of Herbal Centre Individually/Organization Owned

3. If owned by an organization, what type?

……..

4. How many are the members?

……

5. What is your role in it?

……..

6. Can you read and write English? Yes No

7. Name of I/C

……...

8. Location/Address ……...

……

9. Phone number(s)

……...

10. How long have you been practicing herbal medicine?

……...

11. What diseases do you treat at this centre?

……..

12. When did you start treating HIV/AIDS?

……

13. Are drugs for treating HIV itself (……) or for treating the opportunistic diseases (……) or both?

…….

14. How many HIV patients have you treated so far

……

15. What makes you consider somebody a treatable patient?

……..

16. How do you tell that somebody is responding to treatment?

……..

17. How long does it take to complete treatment?

……

18. What do you do to patients after treatment?

……...

19. Is your herbal centre registered? Yes No

20. If yes, with what board?

……..

21. How do you advertise yourself?

……

22. How many HIV patients do you see a) a week …….. b) a month

…….

23. Do you understand the purpose of this questionnaire? Yes No

24. If yes tell us the purpose

…….

25. How did you come by this understanding?

……

26. What do you think of it?

…….

27. Are you and your centre convinced and interested to participate in it? Yes No

28. If necessary will you give out your herbal products for further testing? Yes No

29. If No, why?

……..

30. If requested can you provide your drugs in large/commercial quantities Yes No

31. Will you be willing to sign an informed consent form for us? Yes No

32. If No, why?

……..

33. Would you please give any comments you may have?

……..

……
…

Name …… Sign ……..……. Date…….……

Witnessed by (Name) …….. Sign ……. Date

……..

APPENDIX II

SAMPLE OF QUETIONNAIRE, INFORMED CONSENT FORM AND DATA SHEET USED TO ACQUIRE PROFILE OF PATIENTS

PATIENTS

Evaluating the potentialities of medicinal plants as anti-retroviral therapy against HIV/AIDS

Salutation and pleasantries

Question (Q): Could you please tell us why you are visiting this place?

Answer (A):

Q: How did you get to know this place?

A:

Q: Are you sure you would want to have treatment here?

A:

Q: What do you know about this treatment?

A:

Q: Do you know that this treatment could be hazardous to your health and that it can give you complications and thereby worsen your condition?

A:

It is to make sure that that your condition is not worsened by this drug that is why we are here. As you may know, indeed, herbal medicine has an enviable track record of treating many diseases. However, as you may also know, HIV is incurable and therefore any claim that these herbals can cure it must be supported with facts and evidence. Besides, some of these herbal drugs too can be toxic by themselves and sometimes can aggravate your already not too healthy condition. Our objective here, therefore, is as you go through this treatment, we would like to observe and monitor you to see that nothing harmful happens to you. We will do this by taking a small amount of your blood just before you start taking this drug and there after every month for about six months to go and analyze it in our laboratory to see how this drug is helping you. Apart from taking your blood we will ask you to visit our hospital periodically for our well-qualified doctors there to examine you. If in the course of this monitoring period we see anything detrimental to your health we will tell you immediately and accordingly advice you on the options that you may have. The good news here is that you will not pay anything towards all these; instead we will rather give you some money to help defray some of your transportation costs.

Our main interest here is that we would like to know if indeed this drug can cure your HIV infection, as the owner is claiming so that if it is true we can do further tests to see what is inside it that makes it able to cure HIV/AIDS.

At this point do you have any question(s)? to ask me?

If in time you have any more questions to ask, you may please contact either

Dr T.B. Kwofie or MR P.K. Feglo, both of them at the School of Medical Sciences

(SMS), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi.

If you agree to what we have discussed with you could you please sign the following for us?

INFORMED CONSENT FORM

I, ……., a native of ……..

and aged …….. years wish to attest that the objective and purpose of this study has been thoroughly read and explained to my understanding.

Therefore, I voluntarily and freely agree to participate in this study. I therefore promise that I will strictly adhere and abide to the rules and regulations as outlined to me. If I am ever found to be faulting in any way, I agree to be excluded from this study. I also agree and direct that any information that I will give or obtain from me, including by HIV results status can be used for purposes that have been stated and explained to me.

If, however, the information that I will give here and my HIV screening results are used for any other purpose other than what has been stated here and as explained to me without my explicit consent, that I will reserve the right to take any action against the administrators of this study.

Signature: ……. Date: ……

Witness: ……...

APPENDIX III

PARTICIPANTS' CONFIDENTIALITY

The identity of all participants will completely be anonymous. In fact as soon as a person is admitted to the study as a participant he or she will immediately be given a code. There will be data collection sheets, which will collect other information like age, gender, habitat, occupation and education level. This information will be for statistical and analytical purposes and will and cannot in any way lead to the identification of the participant's identity. Please find a copy of the data collection sheet below.

PATIENT'S DATA SHEET

1. TEST CENTRE……. 2. DATE…….

3. PATIENT'S CODE …… 4. SEX.....

5. AGE.....

6. MARITAL STATUS.....

(i) Married (ii) Single (iii) Divorced (iv) Widow(er)

7. RELIGION (i) Christianity (ii) Islam (iii) Other……...

8. EDUCATION (i) Primary (ii) Secondary (iii) Tertiary (iv) Vocational/Technical

9. HABITAT (i) Rural (ii) Semi-Urban (iii) Urban

10. OCCUPATION

……...

11. HIV STATUS …… TESTED WITH

(KIT)……

12. OTHER CLINICAL SYMPTOMS

…….....