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Immunological Profile of HIV-Infected Patients Undergoing Herbal Treatment

BY

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BSC (HONS) BIOLOGICAL SCIENCES

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IMMUNOLOGICAL PROFILE OF HIV-INFECTED PATIENTS UNDERGOING HERBAL TREATMENT

A THESIS PRESENTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (CLINICAL MICROBIOLOGY)

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DECLARATION

I hereby declare that this thesis is the result of my own original work and that to the best of my knowledge, no part of it has been submitted to any institution or organisation anywhere for the award of a degree.

All inclusions from the work of others have been duly acknowledged.

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May the good Lord reward each one accordingly.

DEDICATION

This thesis is dedicated to my sweet parents Mr. And Mrs. John Sedo-Agbemabiese.

It is also dedicated to my beloved younger siblings Solace and Pearl.

Last but not least, I also dedicate this thesis to my loving and caring husband Mr. Michael Ametepe Kattah.

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ABSTRACT

In a study to evaluate the efficacy of some herbal products as HIV/AIDS therapeutic, three herbal products were assessed in six HIV-infected patients by studying the virological and immunological parameters to see how it may be altered in these patients. CD4+ T – cell numbers, plasma viral load levels, concentrations of IFN –γ and IL – 10 were measured at the point of initiation and then monthly for a period of six months. Patients from Centre one (C001) had a substantial increase in their CD4+ T – cell counts and a significant reductionin their plasma viral load levels by the end of the study indicating the possible efficacy of the herbal product they had taken. Virologically and immunologically patients from Centre two (C002) and Centre three (C003) showed non- response to treatment.

CHAPTER 1

1.0 INTRODUCTION

1.1 THE PROBLEM

The introduction of antiretroviral drugs or Highly Active Antiretroviral therapy (HAART) has enabled the management of Human Immunodeficiency Virus (HIV) infection in patients (Shafer and Vuitton, 1999). However, in developing countries such as Ghana, not every HIV patient who needs this therapy gets it due to its high cost and difficulty in getting it. Besides the inconvenience of taking HAART for the rest of one's life coupling with its adverse side effects, toxicity and emergence of drug resistant viruses make those who are able to get it not able to derive the full benefit of it (Ozsoy and Ernst, 1999). This therefore sometimes leads some people to seek alternate treatment, especially those of herbal medicine base.

Herbal medicines have been known since time immemorial to have antimicrobial and antiviral activities. They have therefore, been used to treat many illnesses and diseases. Whiles herbal medicines have been used to treat many diseases, their efficacy against HIV/AIDS leaves cause to worry particularly as HIV is known to have no cure. Of greater worry is that herbal medicines are not well-researched (Mills, *et al.,* 2005). They are poorly regulated, may sometimes contain compounds that may produce adverse effects in patients taking them. It is even believed that while some HIV-infected patients taking herbal medicines have had some relief from them, others too have had their already worsened conditions aggravated (Ernst, 2002; Melchart *et al*., 1999).

There is therefore, the need to investigate these herbal drugs, and also regulate them such that whatever herbal preparations patients take in will not be harmful to them. (Mills, *et al.,* 2005)

Therapeutic success of any antiviral therapy depends among other things on the monitoring of appropriate laboratory markers. In the case of HIV the markers of plasma HIV RNA (viral load) and $CD4^+$ T cell counts are principally used.

Results of these tests provide evidence regarding the virologic and immunologic status of the patients. Indeed, the demonstration of the prognostic value of $CD4^+$ T cell counts in patients is of greater importance in assessing the immunological response of patients because of its major clinical significance.

Thus this study used $CD4^+$ T cell count together with other laboratory tests, to assess how HIV- infected patients undergoing herbal treatment respond to such treatment. It is to enable us select herbal drugs that may have possible anti- HIV activity for further evaluation.

1.2 JUSTIFICATION

While Traditional Herbal Medicine has been successfully used in the treatment of many diseases and illnesses, their use as a cure for HIV/AIDS is creating worry and anxiety. This anxiety stems from the claims of cure by some traditional medicinal practitioners, despite the currently known incurable nature of HIV infection. This study was therefore, undertaken as means of identifying herbal drugs that may have possible therapeutic effect to HIV and AIDS through the determination of the

immunological parameters that may be altered in HIV/AIDS patients taking these herbal medications.

1.3 AIM AND OBJECTIVE

AIM

- To evaluate the immunological profile of HIV patients undergoing herbal treatment.

OBJECTIVE

- To assess the effectiveness of some traditional herbal preparations in managing HIV/AIDS in patients

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 AN OVERVIEW OF HIV INFECTION

Human Immunodeficiency Virus (HIV) types one(1) and two(2) (Figure 2.1) are the etiologic agents of Acquired Immunodeficiency Syndrome (AIDS) (Brooks, et al. 2004). The illness was first described in 1981 and HIV type one (HIV-1) was isolated by the end of 1983. (Coffin**,** et. al. 1986, Brown, 1991). Since then, AIDS has become a worldwide epidemic, expanding in scope and magnitude as HIV infections has affected different populations and geographic regions. (Joint United Nations Programme on HIV/AIDS, 2006). Infections of HIV are acquired through exchange of HIV – infected body fluids. The virus, once acquired persists indefinitely in infected host, though it may be present at very low levels at some points. The four major routes of transmission are unprotected sexual intercourse, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth.

The human immunodeficiency virus (HIV-1) infects its human hosts through cells expressing CD4 and a coreceptor belonging to the seven-transmembrane G-protein-coupled chemokine receptor superfamily (Berger, *et. al.*, 1999). Macrophage-tropic (M-tropic or R5) HIV-1 variants preferentially target CC chemokine receptor 5 (CCR5)-expressing cells, whereas cells with membrane CXC chemokine receptor 4 (CXCR4) are commonly infected by T- or dual-tropic isolates (T-tropic or X4). Initial transmission, infection, and viral isolates are typically represented by M-tropic HIV-1 variants (Schuitemaker, *et. al.*, 1992; Valentin, *et. al.*,1994), thought to target macrophages and/or dendritic cells (Berger, *et. al.*, 1999). $CD4^+$ T cells (McDougal,

et. al., 1985) along with other putative targets later on harbour mature viruses that through circulation reach lymph nodes and lymphoid organs. Here, they continue to infect immune cells, preferentially $CD4^+$ cells,(Douek, *et. al.*, 2002) in some more vigorous way, as the density of target cells are higher at these places and this infection as well as their destruction later on leads to profound decrease in $CD4^+$ cell count.

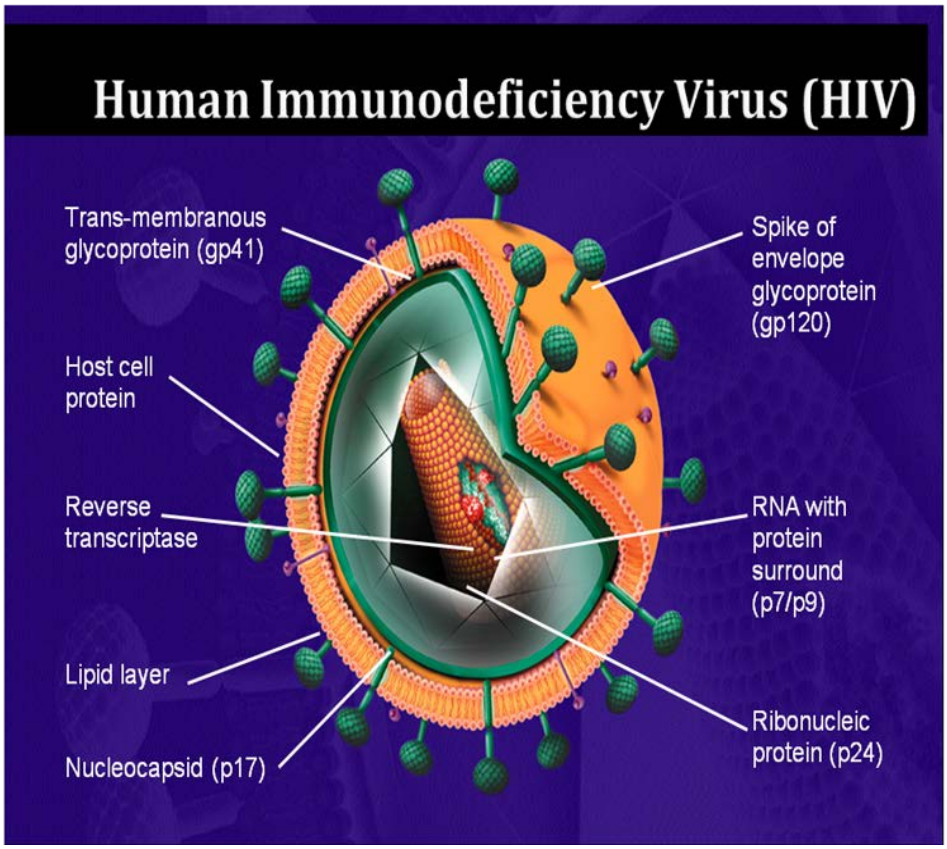

Figure 2.1 Structure of the Human Immunodeficiency Virus (HIV) Source: www.yale edu/bio243/HIV/hivstructure.html

2.2 STAGES OF HIV INFECTION

HIV infection has basically four stages: incubation period, acute infection, latency stage and AIDS. (Figure 2. 2) The incubation period upon infection is asymptomatic and usually lasts between two and four weeks. The second stage, acute infection, is a period of rapid viral replication that leads to an abundance of virus in the peripheral blood with levels of HIV commonly approaching several million copies/ 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.

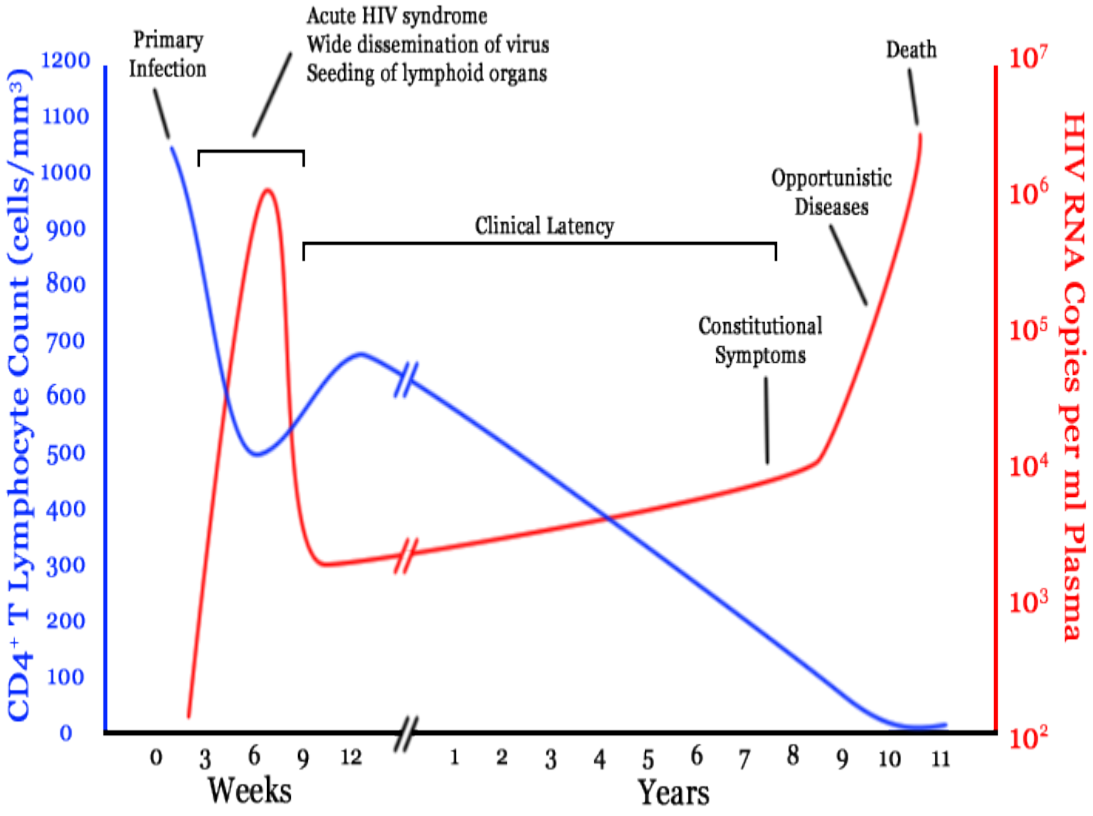

Figure 2.2 Schematic diagram showing the general course of HIV infection
http://en.wikipedia.org/wiki/HIV-timecourse.png

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 about 800 cells/µL (the normal blood value is about 1200 cells/µL). (Kahn, et al. 1998) During this period, most individuals (80 to 90%) develop an influenza or mononucleosis-like illness, the most common symptoms of which may include fever, lymphadenopathy (swollen lymph nodes), pharyngitis (sore throat), rash, myalgia (muscle pain), malaise, and mouth and esophageal sores (Pantaleo, et. al. 1997).

The latency stage, which occurs third, shows few or no symptoms and can last up to twelve years and beyond. AIDS, the fourth and final stage of HIV infection shows as symptoms of various opportunistic infections (Pantaleo, et. al. 1997). The nonspecific nature of the symptoms poses a great challenge to the clinician and underlines the importance of a detailed history of exposure (Cooper, et. al. 1985). AIDS is characterised by pronounced suppression of the immune system and development of a wide variety of severe opportunistic infections or unusual neoplasms (especially Kaposi's sarcoma (Figure 2.3)). With no treatment, the interval between primary infection with HIV and the first appearance of clinical disease is usually long in adults, averaging about 8-10 years. Death occurs about 2 years later (Daar, et al. 2001).

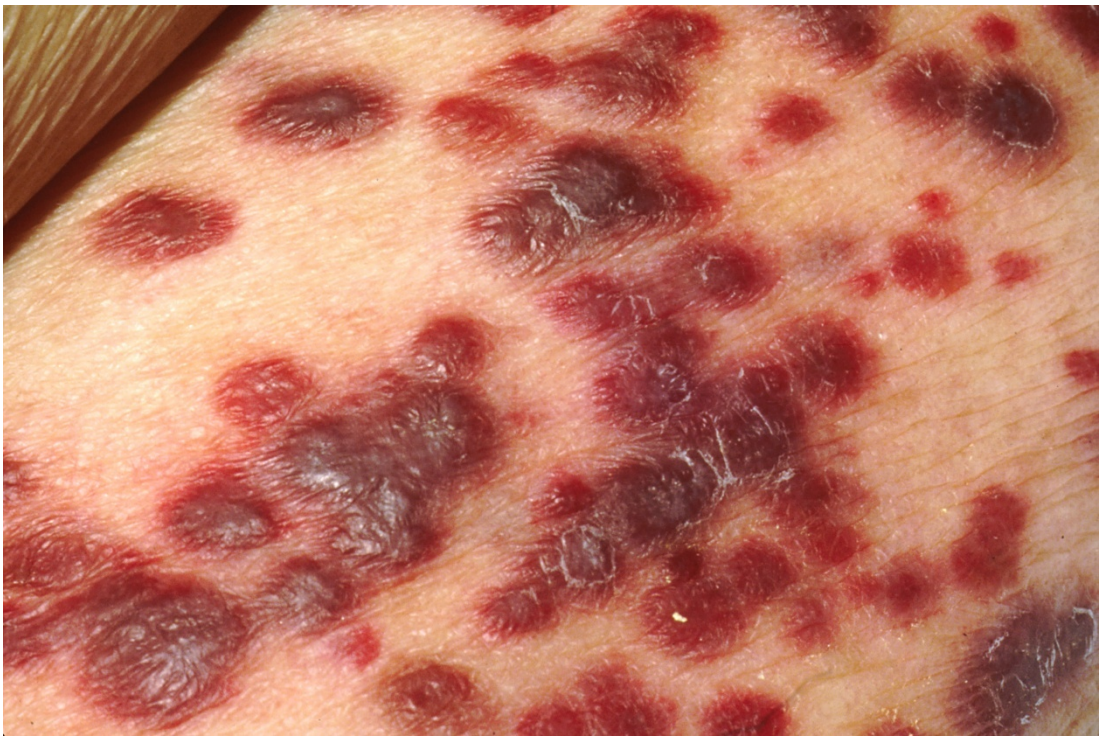

Figure 2.3 Image of Kaposi's sarcoma- one of the opportunistic infections associated with HIV infection Source: http://www.merck.com/mmhe/sec18/ch216/ch216e.html

2.3 THE ROLE OF CYTOKINES IN HIV PATHOGENESIS

Host responses and/or indirect effects of HIV replication are considered to be most important for HIV's ability to undermine the immune system. Cytokines are a heterogeneous group of proteins that are typically secreted in order to exert an effect upon a target cell (Goldsby et al., 2000). The main producers of cytokines are $CD4^+$ T cells and monocytes/macrophages and are differentiated into two distinct subsets, T helper one (Th1) and T helper two (Th2), which were distinguished by mutually exclusive patterns of cytokine secretion in vitro (Mosmann et al., 1986). Th1 cells produce inflammatory cytokines, such as Interleukin two (IL-2) and Interferon gamma (IFN-γ) and these promote cell-mediated immunity while Th2 cells produce cytokines that promote antibody-mediated immunity. Th2 cytokine can either be a B-cell stimulatory factor, such as Interleukin ten (IL-10) or inhibits the activity of Th1 cells (Mosmann & Coffman, 1989; Mosmann & Moore, 1991; Moore et al., 2001).

Infection with the human immunodeficiency virus (HIV) has been shown to induce production of several cytokines both *in vitro* and *in vivo*. Conversely, several cytokines modulate the levels of HIV expression in infected cells of both lymphocytic and mononuclear phagocytic lineage. Mononuclear cells which are always activated in HIV-infected individuals, release HIV-inductive cytokines and thus play a potentially important role in the pathogenesis of HIV infection (Fauci, 1993, 1996; Poli, 1999). Based on measurements of cytokine secretion following in vitro stimulation of peripheral blood mononuclear cells, a hypothesis was introduced suggesting that a shift in the balance of production of Th1 and Th2 cytokines was a major contributor to HIV disease progression (Clerici & Shearer, 1993, 1994). It was proposed that early in HIV infection, a vigorous cell-mediated immune response, facilitated by Th1 cells, effectively controlled the amount of HIV in the body.

However, with time, the predominant cytokine response shifted to a Th2 response, leading to a loss of effective cell-mediated immunity against HIV, permitting increased levels of viral replication, extensive damage to the immune system, and progression to AIDS (Breen, 2002).

Interferon gamma (IFN-γ) one of the Th1 cytokines has been found to be elevated in the plasma, cerebrospinal fluid and lymph nodes of human immunodeficiency virus (HIV)-infected individuals and has shown variable effects on HIV replication in acutely infected cells (Clerici & Shearer, 1993, 1994). IFN-γ has also been shown to inhibit HIV-induced invasiveness of monocytes. (Dhawan *et. al*.,1995). Reuben, (2002) also reports that immune restoration of HIV-1 infected children receiving HAART may be related to an increase in IFN-γ production and a decrease in the rate of IL-10 production after virus suppression. IFN-γ has also been found to inhibit HIV-1 replication in latently infected cells (Jarol *et. al.,* 2002).

Since its description a little more than a decade ago, IL-10, a Th2 cytokine has been the subject of many studies in the context of HIV infection and AIDS, as previous studies have reported discrepant results concerning IL-10 levels during HIV infection. Several studies have shown that IL-10 production is induced *in vivo* and *in vitro* during HIV infection and that neutralisation of endogenous IL-10 may improve defective antigen- specific T cell function in HIV patients (Benjamin *et. al.,* 1992; (Clerici & Shearer, 1994; Barcellini *et. al.*,1994). On the other hand, IL-10 may inhibit T cell apoptosis (Taga *et. al.,* 1993), a potential beneficial effect in these patients. Moreover, while IL-10 has been found to inhibit HIV replication in acutely infected macrophages at concentrations that block endogenous cytokine secretion (Akridge *et. al.,* 1994; Weissman *et. al.,* 1994), lower IL-10 concentrations appear to

enhance HIV replication (Weissman *et. al.,* 1995). Thus, the present data are conflicting and the contribution of IL-10 to the immunopathogenesis of HIV infection remains unclear. Although increased production of IL-10 *in vivo* and *in vitro* has often been reported in association with HIV infection, (Emilie *et al.,* 1990; Fauci, 1993; Graziosi *et al.,* 1994;) there are other reports (Emilie *et al.,* 1997; Breen *et al.,* 2002), suggesting that increased IL-10 *in vivo*, as measured by serum IL-10 levels or *in situ* detection of IL-10 in lymph nodes, is associated not with HIV infection or AIDS in general, but rather, specifically with the development of AIDS-associated non-Hodgkin's lymphoma. While IL -10 may be beneficial when acting as an anti-inflammatory cytokine that suppresses the production of HIV, it can also act as B cell stimulatory factor that could be contributing to the B cell hyperactivity seen in association with HIV infection. (Poli, 1999)

Shin *et al*. (2000) have reported that a genotype for the IL-10 gene that is associated with high IL-10 expression in vitro appears to be protective for progression to AIDS when examining rates of progression 5 years or more after HIV infection. While it is not a direct measure of IL-10 production by cells, this genetic analysis is consistent with the view that higher IL-10 levels are desirable, presumably acting as a suppressor of inflammatory cytokines and HIV replication

2.4 THERAPEUTIC MARKERS OF HIV INFECTION

Infection with the Human Immunodeficiency Virus (HIV) is followed by an asymptomatic period of varied durations characterised by low or absent rate of virus replication, stable or slowly decreasing numbers of $CD4^+$ T-helper cells and qualitative defects in T cell function (El-Sadr *et al*, 1987). Generally, the progressive,

slow, and irreversible destruction of the immune system is not clinically apparent for many years (Tsoukas, 1994). Markers of AIDS development include HIV related symptoms, (Phair, *et al.*, 1990) depletion of CD4+ T cells (Fahey, *et al.*, 1990) elevated serum b2-microglobulin (b2-m) and neopterin levels, (Fahey, *et al.*, 1990) HIV-1 p24 (core) antigenaemia, (de Wolf, *et al.*, 1987) and syncytium inducing HIV-1 phenotype. The most characteristic feature of AIDS is the selective depletion of the $CD4^+$ T cells. According to Tsoukas, 1994, most experimental therapeutic protocols enrol patients on the basis of $CD4^+$ T cell counts, or the presence or absence of viral antigens, or both. These markers of clinical AIDS development are used to make decisions on the timing of medical intervention.

Active replication of virus occurs in all clinical stages of infection. It is possible to detect and quantify virus throughout the course of HIV infection. Plasma viral load (HIV RNA) quantification is presently considered the most representative and sensitive laboratory test for monitoring progression of HIV infection and response to antiretroviral therapy (Dar and Singh, 1999). Measurement of levels of HIV RNA over time have been of great value in delineating the relationship between levels of virus and rates of disease progression, the rates of viral turnover, the relationship between immune system activation and viral replication, and the time to development of antiretroviral drug resistance (Gupta, *et al.*, 2004).

Saag *et al*., 1996 reports that, determination of viral load is essential in the clinical management of HIV infection. In early infection it serves to assess the likely course the infection will take. Based on viral load, appropriate decisions can be made as to when to initiate treatment. The short or long term potency of any antiretroviral

treatment can then be assessed by measuring the reduction in virus concentrations over a period (Saag *et al*., 1996; Mellors, *et. al.,* 1997).

CD4 is one of the several glycoproteins termed "cluster of differentiation (CD) antigens," expressed on the surface of lymphocytes (Malone *et al.,* 1990). Some of these antigens can be used to classify lymphocytes into subpopulations that correlate with their function (e.g. CD3 is expressed on all T cells and CD4 and CD8 on T helper and T cytotoxic lymphocytes, respectively). The most commonly used cellular marker for monitoring progression of HIV infection and for assessing response to therapy is the CD4 lymphocyte count (Fahey *et al.,* 1990). CD4 also serves as a receptor for HIV, and cells expressing this protein usually decline in number with progressive HIV infection. (Gupta, *et al*., 2004) The number of cells that express the CD4 antigen is therefore a useful guide to the pathological effects of HIV on the immune system. Its decline is the hallmark of HIV infection and the rate of loss in each person is unique. $CD4^+$ T cell counts can be measured by flow cytometry, microsphere assay, and Enzyme immunoassay (EIA). Antibodies to these antigens allow a rapid and accurate measurement by flow cytometry of the number of cells expressing each antigen (Fahey *et al.,* 1990). Patients with an initial diagnosis of HIV infection should have $CD4^+$ T cell measurements performed approximately every six months and more frequently if a declining trend is noted (Gupta, *et al.,* 2004).

$CD4^+$ T cell count is extremely important in staging of HIV infection and a revised classification of the centre for disease control (CDC), Atlanta, USA (1993) divides HIV positive persons into three CD4 count categories: 1) >500cells/µL; 2) 200-499cells/µL, 3) <200cells/µL (along with 3 parallel clinical stages A, B, and C). A

low $CD4^+$ T cell counts (less than 10%), a number less than 100/µL and a low CD4/CD8 ratio (<0.2) are highly predictive for death from AIDS-related complications. Many studies of HIV infection have shown a relation between a low $CD4^+$ T cell count and the subsequent development of AIDS (Moss, *et al.*, 1988, Eyster, *et al.*, 1989). Other studies have shown that subjects with low $CD4^+$ T cell counts are at risk of specific AIDS related illnesses such as Pneumocystis carinii pneumonia (Phair, *et al.*, 1990).

The introduction of prophylaxis against various opportunistic infections is based primarily on the $CD4^+$ T cell counts; when to begin antiretroviral therapy is based on both $CD4^+$ T cell counts and viral load estimations. Antiretroviral therapy is generally indicated when the $CD4^+$ T cell count falls below 500/µL. All effective forms of antiretroviral therapy to date have been associated with at least a transient increase in either $CD4^+$ T cell count or CD4 proportion (Malone *et al.*, 1990).

Though $CD4^+$ T cell count is widely used by clinicians, it is a crude predictor of progression of HIV infection. A single abnormal result is not usually a sufficient reason to introduce or change treatment (Malone *et al.*, 1990). It is much more important to follow $CD4^+$ T cell counts serially and to observe emerging trends.

Higher HIV RNA levels correlates with lower baseline $CD4^+$ T cell counts, a more rapid decline in $CD4^+$ T cell counts, and a more rapid disease progression. Patients with more than 100,000 copies/ml of plasma HIV RNA within 6 months of seroconversion have been shown to be 10 fold more likely to progress to AIDS over 5 years than those with fewer copies of plasma HIV RNA (Saag *et al.*, 1996,).

2.5 EFFECTS OF HAART ON HIV

The introduction of potent combinations of antiretroviral therapy or highly active antiretroviral therapy (HAART) in the treatment of HIV-infected patients can effectively suppress HIV replication in a substantial proportion of infected individuals (Gulick, *et al*.1997, Markowitz, *et al*.1999). In addition, HAART has been shown to be beneficial in the reconstitution of T-cell responses and also to augment neutrophils and monocytes function during HIV infection (Lederman, *et al*.2003).

There are different classes of antiretroviral drugs that act at different stages of the HIV life cycle (Figure 2.5). Antiretroviral drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits:

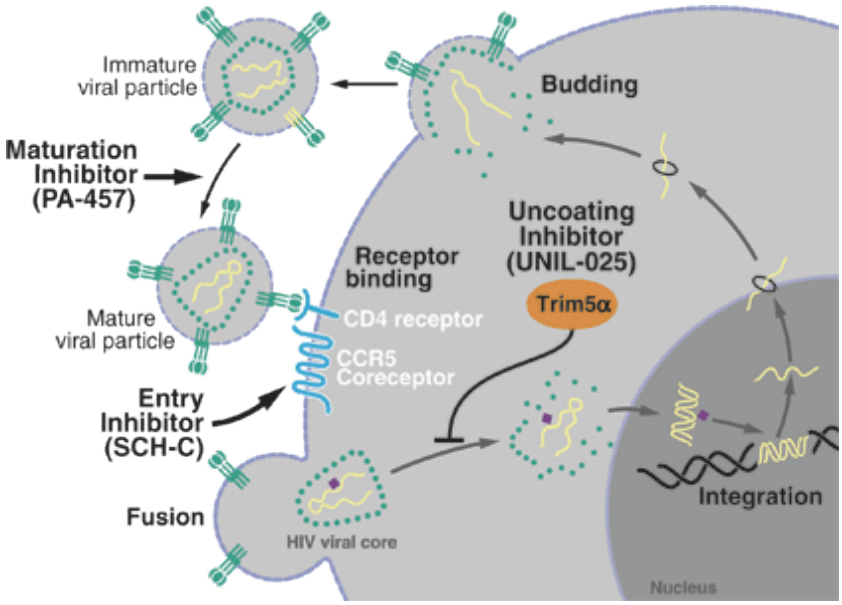

Figure 2.5 Image of possible target sites for antiretroviral therapy in HIV infection. Source: www.gladstone.ucsf.edu/hiv-targetsites

- Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI) inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation (Brinkmann, 1999).

- Non-nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function (Gulick, *et. al.*, 1997).
- Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions (Hammer 1997, Cameron 1998).
- Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two currently available agents in this class (Nelson 2007, Lalezari 2007).

Currently, HAART is the only therapeutic option available for seropositive and symptomatic individuals, and is comprised of targeted inhibitors of HIV-1 reverse transcriptase (NNRTIs and NRTIs) and/or protease (PI) and the newly FDA approved gp41-inhibitor Fuzeon/T20, a fusion inhibitor (Balwin, *et al.* 2003). The current therapeutical strategy, highly active antiretroviral therapy (HAART), involves the use of agents from at least two distinct classes of antiretrovirals (Yeni *et al*., 2004).

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). However, HAART sometimes achieves far less than optimal results, in some circumstances being effective in less than fifty percent of

patients. This is due to a variety of reasons such as medication intolerance/side effects, prior ineffective antiretroviral therapy and infection with a drug-resistant strain of HIV (Becker, *et al*. 2002). However, non-adherence and non-persistence with antiretroviral therapy is the major reason most individuals fail to benefit from HAART. (Becker, *et al.* 2002).

Internationally accepted guidelines for the treatment of HIV-infected patients recommend close monitoring of immune system function, of the patient's viral load and of the virus's resistance to antiretroviral drugs (Yeni, *et al.,* 2002). The laboratory equipment needed to perform such monitoring on a regular basis is costly, complicated to operate and can hardly be envisioned for wide application in resource-poor settings (Yeni, *et al.,* 2002). To that, one needs to add the investments that would be required for the proper use of these technologies: training of personnel, facilities to draw blood from patients, logistics to ensure transport and storage of blood samples, documentation, etc (Bogaards, *et al*. 2003). These requirements, as defined by standard of care in high-income countries, actually represent a significant obstacle to the use of HAART in countries where clean water and electricity are scarce, and where one in four adults is HIV-infected (Bogaards, *et al*. 2003)

2.6 THE USE OF HERBAL DRUGS AS HIV THERAPEUTIC

Since the beginning of human civilization, herbs have been an integral part of society, valued for both their culinary and medicinal properties (Vickers, *et al.* 1999). Herbal medicine has made many contributions to commercial drug preparations manufactured today including ephedrine from *Ephedra sinica* (ma-huang), digitoxin from *Digitalis purpurea* (foxglove), salicin (the source of aspirin) from *Salix alba*

(willow bark), and reserpine from *Rauwolfia serpentine* (snakeroot), to name just a few. (Mashour, *et. al.* 1998). The use of plants for healing purposes predates human history and forms the origin of much modern medicine (Vickers, *et al.* 1999). Plant products are perceived to be healthier than manufactured medicines (Corbin, *et. al.*, 1998).

The safety and effectiveness of most alternative medicines have not been scientifically proven (Talalay, *et al.*, 2001) and remain largely unknown (Eisenberg, 1991). Elvin-Lewis, (2001) reports that, adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal. Herb-drug interactions are a concern; numerous herbs are known to have interactions with drugs (Elvin-Lewis, 2001).

In the context of HIV treatment in Africa, patients often choose traditional healing systems as primary care for HIV- related problems including dermatological disorders, nausea, insomnia and weakness (Peters, 2004, Homsy *et al.*, 2004). A clinical evaluation of an unspecified traditional herbal medication by Tshibangu *et al.* (2004) showed some interesting results. Within eight months of therapy, there was a significant health improvement with regard to physical appearance, disappearance of urogenital lesions, weight gain, as well as a significant reduction in viral load and a significant increase in $CD4^+$ T-cell count. However, herbal medicines can cause psychiatric and neurological adverse effects due to improper use, intrinsic toxicity of ingredients, contamination and adulteration of preparations, interactions with conventional drugs (Ernst, 2000).

Notwithstanding these concerns, the use of traditional medicines by Africans living with HIV is believed to be widespread, although insufficiently documented (MacPhai *et al.,* 2002; Sebit *et al.,* 2000; Zachariah *et al.,* 2002). Although there is not enough evidence on effectiveness, and the possibility of harm, the Ministries of Health of some African countries currently promote traditional herbal medicine for the treatment of HIV and associated symptoms (SADC, 2002; Morris 2002). The need to monitor the progress of treatment of patients undergoing herbal therapy is therefore necessary to ensure that their already deteriorating conditions are not worsened by the drugs.

CHAPTER THREE

3.0 METHODOLOGY

3.1 HERBAL THERAPY CENTRES

Traditional herbal medicine practioners from six herbal centres were contacted for our study. These are

- M and Jay Management Consult, Mampong
- Obeng Memorial Herbal Clinic, Pankrono, Kumasi
- Amansa Boafo Herbal Centre, Kronum New Site, Kumasi
- Tawheed Naturopathic Clinic, Boadi, Kumasi
- Akobalm Herbal Enterprise, Sepetimpom, Kumasi
- Yehowa Behwe Enterprise Atwiwa- Brofoyedru, Kumasi.

These herbal centres were known for the treatment of patients with various diseases and also claimed to treat HIV/AIDS patients as well. Information about the herbal centres including location, number of years of practice, diseases treated and information about their HIV/AIDS drugs were taken after the herbalist had completed a designed form and questionnaire (Appendix 1) regarding the profiles of their respective herbal centres and drugs. All centres were located in the Ashanti Region of Ghana and had had a considerable number of years of practice.

The herbal centres were to provide their own patients who must be willing to take their drugs. Only three herbal centres, listed below could meet the full demands of the study. These centres and their herbal preparations were selected for the study.

- M and Jay Health Management Consult

- Obeng Memorial Herbal Clinic
- Amansa Boafo Herbal Clinic

Codes (001-003) were assigned to the selected herbal centres by which they were identified. Thus

- C001- M and Jay Health Management Consult
- C002 – Obeng Memorial Herbal Clinic
- C003 – Amansan Boafo

The profiles of these centres is summarized in Table 3.1

Table 3.1 Profile on the three Herbal Centers

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	Organisation	Individually owned	Individually owned
Location address	Mampong	Kumasi	Kumasi
Years of practice	11 years	18 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	1999	1992	1994
Other diseases treated by the HIV/AIDS drug	None	Broad spectrum of infections and cancers	Asthma, whites, chronic diarrhoea, breast cancer, eye disease
Number of HIV patients treated so far	720	26	100 and over
Criteria for patients HIV treatment	If one can guarantee accessibility to nutritional support and to control secondary infections.	When CD4 normal levels is stable without drugs for 6 months	The symptoms associated with HIV
How response to treatment is monitored	CD4 cell count, LFT& RFT PRN positive change in appetite if clinically symptomatic	Through laboratory results and or when opportunistic disease declines	When symptoms are vanishing
Duration of the treatment process	6 months, but WHO category 4 as long as possible	6 to 12 months	Between 6 and 8 months

3.2 HERBAL PREPARATIONS

The three selected herbal centres produced the herbal preparations that were evaluated in this study. These preparations were purported to be anti- HIV therapeutic. Table 3.2 gives the descriptions of the herbal preparations used in the study.

Table 3.2 Profile on the praparations administered to patients by the herbalist

DRUG PROFILE	CENTRE 1	CENTRE 2	CENTRE 3
Name of herbal preparation	MJ GOLDONI	Misparon OA, Unity mixtures	Amansa Boafo
How potency of preration was realised	Changes in CD4 counts morphological & appetite changes in clinically symptomatic clients	Through invivo & in vitro test and clinical evidence	After trying it on some HIV positive patients recommended by doctors
Nature of Preparation	Liquid	Liquid (water in oil)	Like tonic
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	Stabilised with sodium benzoate, keep in cool place	None	Mixed sodium benzyl
Shelf-life	One and half years	Three years	Three years
Administration	Orally	Oral and topical	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

3.3 STUDY SUBJECTS

Six study subjects, comprising three males and three females, who were all HIV positive and had been treatment naïve for antiretrovirals, were provided by the three selected herbal centres and were enrolled into the study as study subjects. Laboratory tests were performed to confirm their HIV status. The subjects were then requested to complete a designed form (Appendix 2) consisting of a questionnaires, an informed consent form and a data sheet about their background. The baseline characteristics of subjects (Table 3.3) were acquired from this and codes were then assigned to each of them by which they were identified.

Table 3.3 Profile of study subjects

Patient's ID	**Centre Code**	**Patient's Code**	**Sex**	**Age**	**Marital Status**	**Education**	**Religion**
C001/P01	C001	P01	F	31	Single	Primary	Christianity
C001/P02	C001	P02	F	41	Divorced	Primary	Christianity
C001/P03	C001	P03	M	45	Divorced	Secondary	Christianity
C002/P01	C002	P01	M	40	Married	Primary	Christianity
C002/P02	C002	P02	F	38	Married	None	Christianity
C003/P01	C003	P01	M	34	Single	Primary	Islam

Table 3.3 continued

PATIENT'S 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 diarrhea stage 3
C002/P01	Low income	Semi-Urban	Positive	Chronic diarrhea stage 3
C002/P02	Low income	Semi-Urban	Positive	-
C003/P01	Low income	Rural	Positive	Oral candidiasis stage 3

Prior to the inception of this study, the clinical and physical conditions of subjects were assessed by a qualified medical practitioner upon consultation after which a sample of venous blood was taken for laboratory tests. The subjects were then put on the herbal drugs by their herbalists. They were made aware of the fact that they need to adhere to their scheduled regimen of the herbal preparation and were also given time for regular visits throughout the study period. Assessment of subjects' clinical and physical conditions and blood sampling were continued monthly over a six month period. During every data collection period, the HIV status of subjects was checked to ascertain whether there could be any change.

3.4 INCLUSION CRITERIA AND EXCLUSION CRITERIA

3.4.1a Inclusion criteria for selection of herbalists

- To be eligible, a herbalist has to be a registered member of Traditional and Alternate Medical Association and also recognized by their community.
- Herbalist must be able to recruit their own study subjects.
- Herbal preparation must have been tested to be safe for human consumption by the Mampong Centre for Scientific Research into Plant Medicine.

3.4.1b Exclusion criteria

- Herbalists who are not able to recruit their own study subjects
- A herbalist whose preparation have not been approved by Mampong Centre for Scientific Research into Plant Medicine to be safe for human consumption.

Herbalists would have to comply with study requirements before partaking in the study.

3.4.2a Inclusion criteria for patient selection

- Patient must be 18years and above.
- Patient must have CD4 count below 250 or WHO stage 3.
- Patient must be antiretroviral naïve.
- Patient must be willing to participate.

3.4.2b Exclusion criteria for patient selection

- Persons who are HIV negative
- Persons who are below 18years
- HIV infected patients who are on treatment
- Patients who are unwilling to participate

Study subjects must meet the above mentioned requirement before undergoing the study.

3.5 ETHICAL MATTERS

A designed informed consent forms were signed by subjects indicating their voluntary participation in this study. They also agreed to abide by the regulations governing this study. The informed consent also gives the right to the subject to take any action against the administrators of the study in case of any breach in agreement.

3.6 LABORATORY TESTS

Laboratory tests were carried out at the point of initiation of therapy and there after monthly for over a six month period. Tests included:

HIV Antibody response of patients

Patients' chemistry and haematology profiles

Patients' $CD4^+$ T cell and HIV RNA copies counts

Patients' circulating cytokine profile

3.6.1 BLOOD SAMPLING

Ten (10ml) of venous blood samples were collected from each patient of which 6ml was dispensed into ethylenediaminetetraacetic acid (EDTA) containing tubes (Vacutainer, BD Biosciences, San Jose, CA, USA) and 4ml into plain pyrogen free tubes (Greiner Bio-One, Bad Hal Kremsmuester, Austria). Some of the blood sample in the EDTA tube was used for HIV antibody screening using (Determine HIV - 1/2 antibody test (ABBOT, Minato – ku, Tokyo, Japan)), hematological parameters and $CD4^+$ T cell count. Plasma was extracted from the remaining anticoagulated blood, aliquoted and stored at -80ºC. Some of this stored plasma was used for HIV RNA copies determination.

Blood samples in the plain tubes were centrifuged and serum was obtained. Some of the serum was used for HIV antibody test by OraQuick HIV 1/2 Antibody test kit (Orasure Technologies, Bethlehem, PA, USA) and also for clinical chemistry test. The rest of the serum was aliquoted into 100µl plain tubes and stored at -80ºC and assayed for IFN- γ and IL – 10. Samples were frozen and used once.

3.6.2 HIV ANTIBODY RESPONSE

Rapid HIV antibody response testing was conducted using Determine-HIV 1⁄2 assay kit and was confirmed using OraQuick HIV-1/2 Rapid Test.

Determine HIV 1/2 Antibody test: This is an immunochromatographic test which is visually read. Each test kit comprises of a patient window labelled P and a control window labelled C. A red bar at the patient's window indicates the presence of antibodies to HIV. A test is said to be valid if the bar at the control window turns red.

With the Determine-HIV 1⁄2 assay kit (Figure 3.1a), about 50µl of plasma was dropped into the sample port of the rapid test device after which the sample moved across the test bar. The result was read between 15 and 60 minutes.

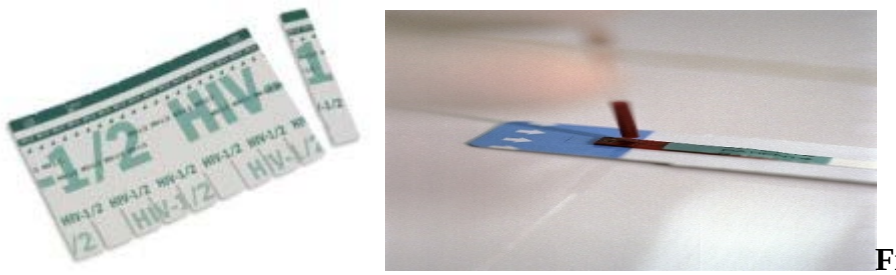

Figure3.1a

Image of unused and used Determine HIV 1/2 kit from ABBOT Laboratories. Source: Personal collection

OraQuick HIV1/2 test: The OraQuick test kit (Figure 3.1b) comes in the form of vial containing reagent and a dip stick. This test is also an immunochromatographic test for the detection of antibodies to HIV 1/2 in whole blood, plasma, serum, or human oral fluid. With this test kit a drop of serum was added to the reagent in each vial using Pasteur pipette and the dip stick was then put in. The reagent- sample mixture rises by capillary action and the test result is read in about 20 to 40 minutes.

Figure 3.1b: Image of OraQuick HIV test kit from OraSure Technologies. Source: Personal collection

3.6.3 CHEMISTRY TEST

The biochemical profiles of study subjects were assessed using BT 3000 PLUS (Biotecnica Instruments, Licenza, Rome, Italy (Figure 3.2)) which is an analyzer for clinical chemistry and immunoturbidity. The BT 3000 PLUS is equipped with software that offers maximum flexibility in the performing of tests on serum, plasma and urine. 100µl of serum was analyzed for the liver enzymes such as aspartate amino transferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and also for total bilirubin, creatinine and Blood Urea Nitrogen (BUN)

Figure 3.2 BT 3000 PLUS. Source: Personal collection

3.6.4 HAEMATOLOGY

Blood in EDTA containing tubes was processed within two hours of handling for haematology. Haemoglobin levels, the quantification of platelets and of white blood cells and differential counts were done using the Auto Haematology Analyzer, BC - 3000 Plus (MINDRAY, Nanshan, Shenzhen, China). This is an automated haematology analyzer that provides information on white blood cells, differential and also on reticulate analysis. In this test, each whole blood sample was mixed thoroughly by gentle agitation and then fed to an aspirator on the BC- 3000 machine after which results were produced and printed out within a minute.

3.6.5 CD4 T-CELL ENUMERATION

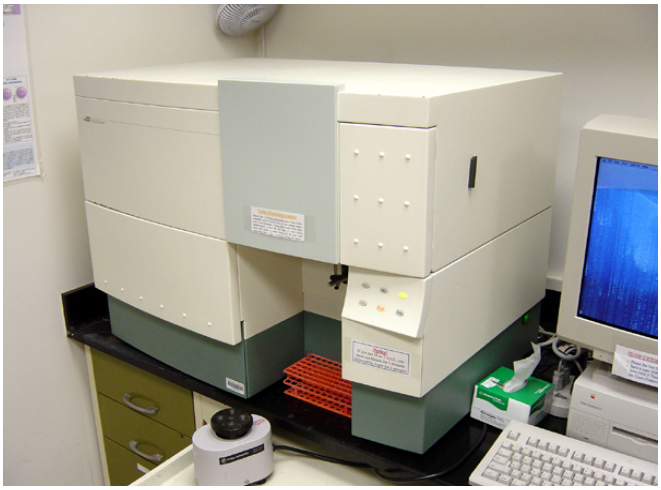

Figure 3.3 BD FACSCalibur System. Source: Personal collection

Blood in EDTA containing tubes was processed within 4 hours of handling for the enumeration of $CD4^+$ T cell numbers. $CD4^+$ lymphocyte numbers in whole blood was assessed using four color flow cytometric analysis with BD FACSCalibur System (BD Bioscciences, San Jose, CA, USA). This is a bench top, flow cytometer system capable of both analysing and sorting cells. The machine as shown in Figure 3.3 is also equipped with a FACStation computer and an optional automated sample loader. Whole blood lysis procedure and BD MultiTEST reagent (BD Biosciences, San Jose, CA, USA) were used in the test.

20µl of MultiTEST reagent was pipetted into TruCOUNT tubes (BD Biosciences, San Jose, CA, USA) labelled according to the samples. 50µl of well mixed whole blood samples were then pipetted into their corresponding tubes which were agitated using a vortex. Tubes were incubated in the dark at room temperature for 15 minutes after which 450µl of FACS lysing solution was added to each tube and the mixture was again agitated. After further incubation of tubes in the dark for 15 minutes, the tubes were again vortexed to reduce aggregation. These were fed into the automated sample

loader on the FACSCalibur system. The samples were run to identify lymphocytes and sub lyphocytes using the appropriate software on the FACStation computer. The CD4 lymphocyte count was recorded for each sample from the results obtained.

3.6.6 MEASUREMENT OF HIV RNA COPIES IN PLASMA:

Figure 3.4 COBAS AMPLICOR Analyzer. Source: http://molecular.roche.com/platforms/cobas_amplicor_analyzer.html

The COBAS AMPLICOR HIV-1 MONITOR test version 1.5 (v 1.5) from Roche Molecular Systems, Branchburg, NJ, USA, in Figure 3.4 was used to quantify plasma viral load. This is a nucleic acid amplification test for the quantification of HIV – 1 RNA in human plasma for use on the COBAS AMPLICOR Analyzer. This instrument fully automates the amplification and detection procedure of the polymerase chain reaction (PCR) process. Included in the Amplicor HIV – 1 Monitor test kit is the Quantitation Standard RNA and Master Mix reagent. The test to quantify HIV – 1 RNA over a range 400 -750,000 copies /ml is based on 5 different steps as follows;

1. Specimen preparation during which target HIV – 1 RNA was isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol.

2. Reverse transcription of target RNA to generate specific complimentary DNA (cDNA).

3. Polymerase Chain Reaction (PCR) amplification of target cDNA using HIV -1 specific complimentary primers, SK145 and SKCC1B.

4. Hybridisation of the amplified products to oligonucleotide probes specific to the target.

5. Detection of the probe – bound amplified products by colometric determination. The absorbance was measured by the COBAS AMPLICOR Analyzer at a wavelength of 660nm.

Quantitation of HIV- 1 viral RNA was done using Quantitation Standard RNA. The HIV- 1 Quantitation Standard is a non infectious RNA transcript that contains the identical primer binding sites as the HIV – 1 RNA target and a unique probe binding region that allows Quantitation Standard amplicon to be distinguished from HIV- 1 amplicon. The HIV – 1 Quantitation Standard was incorporated into each individual specimen at a known copy number and was carried throughout the test process along with the HIV – 1 target and was amplified together with it. The COBAS AMPLICOR Analyser then calculated the HIV – 1 RNA levels in the test specimens by comparing the HIV – 1 signal to the Quantitation Standard signal for each specimen.

3.6.7 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF IFN-γ AND IL-10 LEVELS IN SERUM

The determination of IFN-γ and IL-10 levels in cell-free serum was done using a commercially available ELISA kit (ELISA Ready-SET-Go! eBioscience) according to the manufacturer's instructions. The Human IFN-γ and IL-10 ELISA kit contained the needed reagents, buffers and diluents for carrying out quantitative enzyme linked immunosorbent assays. The IFN-γ ELISA had a sensitivity of 4pg/ml with a standard curve range of 4-500pg/ml, whereas IL-10 ELISA had a sensitivity of 2pg/ml and a standard curve range of 2-300pg/ml. Each ELISA kit consisted of the following:

- Capture Antibody (Pre-titrated, purified antibody)
- Detection Antibody (Pre-titrated, conjugated antibody)
- Standard (Recombinant cytokine for generating standard curve and calibrating samples)
- ELISA/ELISPOT Coating Buffer which was reconstituted into a liquid form of 1L using deionised water and filtered using 0.22µm non-pyrogenic sterile fibres.
- Assay Diluent (5x concentrated)
- Detection enzyme (pre-titrated Avidin HRP)
- Substrate solution (Tetremethylbenzidine Substrate Solution)
- Certificate of analysis (Lot specific instructions for dilution of antibodies and standard.

Separate assays were carried out for the quantitative determination of IFN-γ and IL-10 levels. Fisher 96- well ELISA plates (Fisher Scientific Pittsburgh, PA, USA) were coated with 100µl/well of Capture Antibody in coating buffer. The plate was sealed and incubated overnight at 4^{o}C. Wells were aspirated and washed manually 5 times

with >250µl/well wash buffer of 1x Phosphate Buffered Solution (PBS) containing 0.05 % Tween - 20. Wells were then blocked with 200µl/well of 1X Assay Diluent and plates were incubated at room temperature for 1 hour. Wells were aspirated and washed as was done above after which 100µl/well of standards were added to appropriate wells. 2-fold serial dilutions of the top standards were performed to make the standard curve. 100µl/well of sample was added to the appropriate wells. The plates were then sealed and incubated at room temperature for 2 hours. This was followed by another washing step. A 200µl/well of detection antibody diluted in 1x Assay Diluent was added to the wells after which the plates were sealed and incubated at room temperature for an hour. The plates were again washed and100µl/well Avidin – HRP diluted in 1x Assay Diluent was added to each well. The wells were washed after incubating the plates at room temperature and 100µl/well of substrate solution was then added to each well. The plates were sealed and incubated at room temperature for 15minutes and then 50µl of stop solution of 1M H_3PO_4 was added to each well. The plates were read at 450nm using an automated microplate ELISA reader, Multiskan EX from Thermo Fisher Scientific, Waltham, USA. From the results obtained a standard curve was run on each assay plate using Microsoft Office Excel 2007. The concentrations of IFN-γ and IL-10 in the samples were calculated by interpolation from a standard curve.

CHAPTER FOUR

4.0 RESULTS

4.1 PROFILE OF HERBAL CENTRES

4.1.1 M AND JAY HEALTH MANAGEMENT CONSULT

This is an organisationally run centre, located in Kumasi in the Ashanti Region of Ghana. They have been treating a number of illnesses including hepatitis, buruli ulcer and HIV/AIDS for over a period of 8 years. This centre started the treatment of HIV/AIDS in 1999 with the herbal drug, MJ GOLDONI and has treated about 720 HIV/AIDS patients as of the time of our study. HIV treatment last a period of six months, of which a successful treatment is assessed by an increase in $CD4^{+}$ T cell numbers and Liver Function Test.

4.1.2 OBENG MEMORIAL HERBAL CLINIC

Obeng Memorial Herbal Clinic, also based in Kumasi is owned by an individual. This centre treats a number of diseases which includes HIV/AIDS. They have been operating for 12years and began treatment of HIV/AIDS in 1992. The herbal drug used in the treatment of HIV/AIDS is known as Misparon OA Unity Mixtures which is also used in the treatment of other infections and cancers. Twenty- six (26) patients have so far been treated with this mixture, and treatment lasts for 6 to 12 months. Treatment is evaluated by monitoring declines in opportunistic infections.

4.1.3 AMANSA BOAFO HERBAL CENTRE

This herbal centre is owned by an individual and also based in Kumasi and has been practicing for 14 years. They are known for the treatment of HIV/AIDS and other diseases. Treatment of HIV/AIDS began in 1994 with Amansa Boafo as the herbal preparation. They have treated over 100 HIV patients. Treatment usually last for a period of 6 to 8 months and is assessed by monitoring declines in symptoms.

4.2 PROFILE OF HERBAL PREPARATIONS

4.2.1 MJ GOLDONI

This herbal preparation produced by M and Jay Health Management Consult is made from the parts of some medicinal plants using the method of water extraction. It is then stabilised with sodium benzoate and kept in a cool place. It is administered orally and has a shelf life of one and half years. It has known side effects which includes suppression of appetite and hepatotoxicity if misused.

4.2.2 MISPARON OA UNITY MIXTURES

This is a liquid mixture produced from the roots and stem bark of some medicinal plants by the essence oil extraction method. This herbal drug has a shelf life of three years and 45ml of it is administered orally. Misparon OA Unity Mixtures from Obeng Memorial Herbal Clinic has no known side effects.

4.2.3 AMANSA BOAFO

This is a product of Amansa Boafo Herbal Centre which is a liquid mixture prepared from the parts of some plants by the water extraction method. This mixture has a shelf life of three years which was stabilised by adding sodium benzyl to it. Patients are required to take one tablespoonful, three times daily. Amansa Boafo has no known side effects.

4.3 CHARACTERISTICS OF STUDY SUBJECTS

All patients used for the study had tested positive for HIV and were naïve for any antiretroviral treatment prior to enrolment. Of our six study subjects, four (C001/P02, C001/P03, C002/P01 and C003/P01) successfully completed the program. In the middle of the study period, patient C001/P01, a female aged 31years, died while patient C002/P02 who was also a female aged 38 years had to drop out of the program.

M and JAY Health Management Consult recruited patient C001/P02 who is a 44 year-old trader, a divorcee and a Christian and also dwells in an urban community along with a 45 year old male patient C001/P03 also divorced. This patient is also a Christian and dwells in a semi urban community. Patient C002/P01, recruited by Obeng Memorial Herbal Clinic is 40 years old and is married and dwells in a semi-urban community. He is also a Christian. A 35 year old male patient C003/P01 is the only patient from Amansan Boafo Herbal Clinic and is single. He is of the Islamic faith, lived in a rural settlement and had never been married.

The subjects who successfully completed the study had their education up to the primary school level with the exception of patient C001/P03 who had his education up

to the secondary school level. Additionally all the four subjects were low income earners.

4.4 INTERPRETATION OF RESULTS

4.4.1 CD4 $^{+}$ T CELL COUNT

From Figure 4.1, a general increase could be seen in CD4 values for patients from centre one (C001).

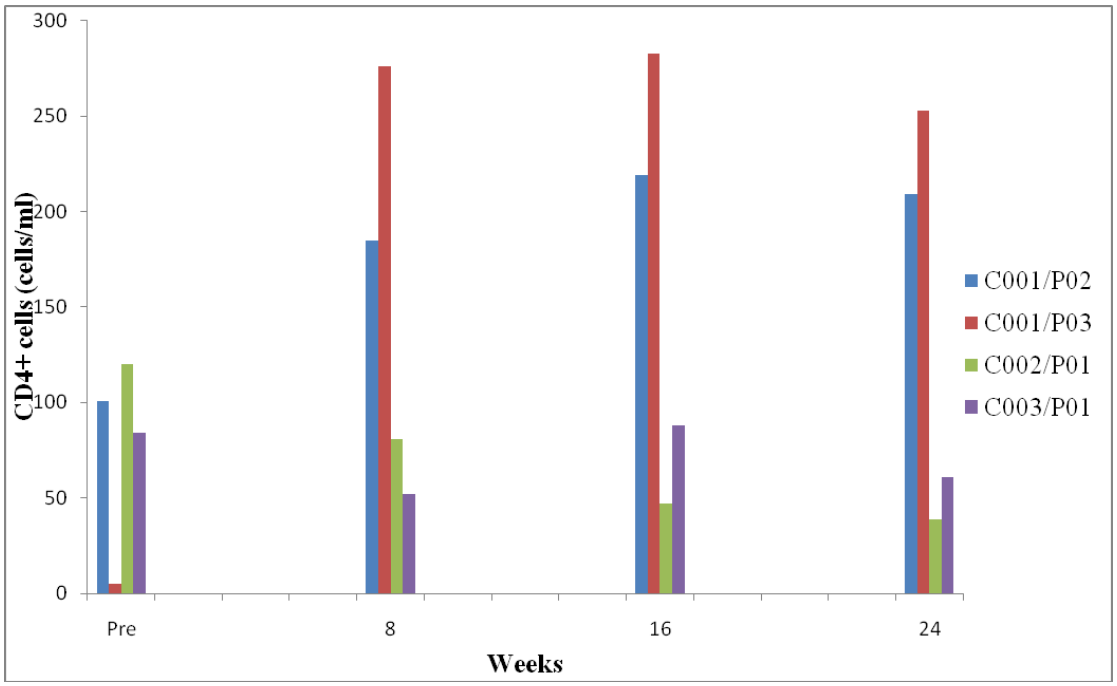

Figure 4.1 A graph showing CD4 counts of study subjects at the various time points

As shown in Figure 4.1, C001/P03 had the lowest CD4 count of 5cells/µl at baseline but at the end of the study had the highest count (253cells/µl). In contrast, C002/P01 had the highest CD4 count of 120cells/µl at the start of study but surprisingly, this count reduced to 25cells/µl at the end of the study. The figure also shows a steady

increase in CD4 cell count for C001/P02 who started with a baseline count of 101 cells/µl shot up to185 cells/µl after 8weeks and ended up with 209cells/µl by the 24th week of study. Again it was observed that, C001/P03 had a baseline CD4 cell count of 5 cells/µl which by the 8th week rose to 276cells/µl, shot up to 283cells/µl by week 16 and finally by the 24th week was at 253cells/µl.

At the start of the study, the only patient from centre two, (C002/P01) had a baseline CD4 count of 120cells/µl, which was the highest baseline value. This could be seen in Figure 4.1. The count decreased to 81cells/µl by week 8 and finally to 25cells/µl by the 24th week. Again from Figure 4.1 it can be observed that there was no specific pattern for the CD4 cell count for C003/P01. At baseline, the count was 84cells/µl and then decreased to 52cells/µl after 8 weeks. After 16 weeks it rose to 88cells/µl which finally decreased to 61cells/µl by the 24th week

4.4.2 PLASMA VIRAL LOAD

As shown in Table 4.1, C001/P02 had a baseline value for viral load of 9.4 x 10^5copies/ml at the start of study but interestingly by the 8th week was undetected. (Detection level > 400copies/ml).

With C001/P03 also in Table 4.1, there was a decline in viral load from a baseline value of 8.49 x 10^5copies/ml to 1130 copies/ml by the 8th week and was undetected for weeks 16 and 24.

C002/P01 had a lower baseline value viral load as compared with the patients from centre one (C001). The baseline value which was 3.2 x 10^5copies/ml decreased slightly after 8 weeks to 3.13 x 10^5copies/ml and moved up to 8.03 x 10^5copies/ml by the 24th week.

For C003/P01, the value for baseline was 3.13 x 10^6copies/ml by the 8^{th} week decreased to 1.14 x 10^6copies/ml. At week 16 the value increased to 1.85 x 10^6copies/ml and by the 24^{th} week reduced to 1.22 x 10^6copies/ml. (Table 4.1)

Table 4.1 Plasma viral load levels

	Treatment Weeks	C001/P02	C001/P03	C002/P01	C003/P01
Plasma Viral Load (copies/mL)	PRE	940000	849000	320000	3130000
	8	< 400	1130	313000	1140000
	16	< 400	< 400	252000	1850000
	24	< 400	< 400	803000	1220000

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4.4.3 CYTOKINE LEVELS

For C001/P02, the values for IL-10 and IFN-γ were 179pg/ml and 296pg/ml respectively at baseline which then decreased to 129pg/ml and 151pg/ml. By the 16^{th} and the 24^{th} week both values were at zero. (Figure 4.2)

It can be seen in Figure 4.2 that, C001/P03 had 410pg/ml and 273pg/ml for IL-10 IFN- γ respectively at baseline. By the 8^{th} week both values reduced to 310pg/ml and 196pg/ml respectively. It was observed that by the 16^{th} week, IL-10 rose to 390pg/ml and shot to 775pg/ml by 24^{th} week while IFN- γ reduced to 162pg/ml at week 16 and totally receded by the 24^{th} week

C002/P01 had no value for IL-10 but had 73pg/ml for IFN- γ as baseline values. By week 8 these values rose to 129pg/ml and 684pg/ml for IL-10 and IFN- γ respectively. By the 16th week IL-10 shot up to 715pg/ml while IFN- γ reduced to 410pg/ml. IL-10 slightly reduced to 710pg/ml with IFN- γ also reducing to 280pg/ml by the 24th week as shown in Figure 4.2.

In the same Figure 4.2, C003/P01 had 530pg/ml as baseline value of IL-10 which almost doubled to 1025pg/ml by week 8 while the baseline value for IFN- γ was 250pg/ml which increased to 260pg/ml. The 16th week shows a reduction in the values for both IL-10 and IFN- γ. IL-10 further reduced to 575pg/ml while IFN- γ increased to 410pg/ml by the 24th week.

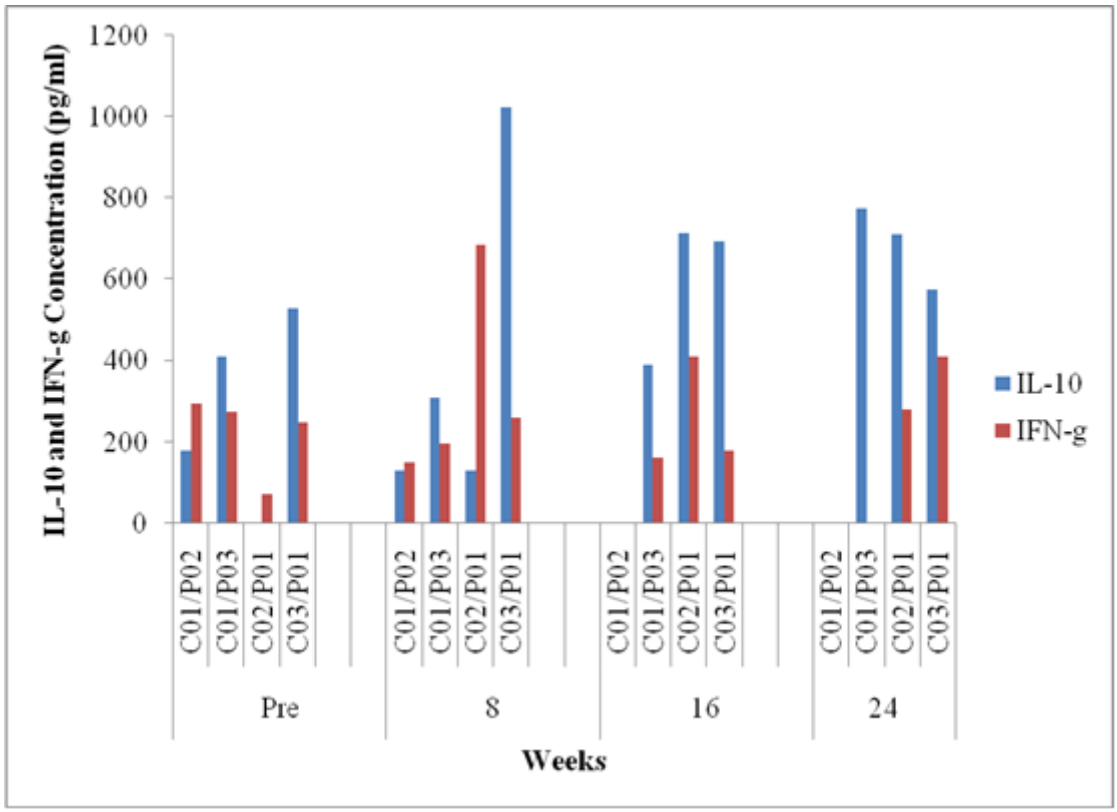

Figure 4.2 A graph of IL-10 and IFN- γ in pg/ml at the various time points in weeks

4.4.4 HAEMATOLOGICAL PROFILE

As shown in Table 4.2, all patients have their platelet counts falling within the reference range and at baseline only the haemoglobin and hematocrit values for patientC002/P01 were within the reference range. All the patients had their white blood cell (WBC) counts within the reference range with the exception of patient C001/P02. Assessment of the differential white cell count revealed that, the percentage of granulocytes (Gran) was within the reference range for patient C002/P01 while the other patients recorded lower values. The percentage of lymphocytes (Lym) was high for patients C001/P02 and C001/P03 with the others recording normal values. With regards to the percentage of mid cells (Mid) which

consist of monocytes, eosinophils and basophils, patient C001/P03 was within the normal range while the others recorded higher percentages.

Haemoglobin and hematocrit values for patients C001/P02 and C001/P03 became normal by the end of the study whereas that of the other two fell outside the range. Patients C002/P01 and C003/P01 attained normal granulocyte and lymphocyte counts whereas patients C001/P02 and C001/P03 had lower granulocyte and higher lymphocyte percentages. However, only patient C001/P03 had a normal percentage of mid cells of 7.4% with the other patients having higher values.

Table 4.2 Table of Haematological Parameters of study subjects

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

4.4.5 BIOCHEMICAL PROFILE

Table 4.3, shows the clinical chemistry results of patients, with normal total bilirubin values which remained unchanged by the end of the study period. With the exception of patient C003/P01, all patients had normal Blood Urea Nitrogen (BUN) levels which remained at reference range at the end of the study. The creatinine level of patient C001/P02 at the point of collection was within the reference range and remaining so at the end of the study period. The remaining patients had their creatinine levels outside the normal range.

With respect to the liver enzymes, only patient C001/P03 had his aspartate aminotransferase (AST) level falling outside the reference range at the point of intiation, and remained the same at the end of the study period. Patients C001/P02 and C002/P01 had normal values of gamma-glutamyl transferase (GGT) at the start of the study with patient C001/P03 recording very high values which remained so by the end of the study. Patient C003/P01 also had high GGT values at the start of the study, but managed to attain a normal value. However, the level of GGT fell outside the normal range for patients C001/P02 and C002/P01 by the end of the study.

All patients maintained normal aspartate aminotransferase (AST) levels throughout the study period with the exception of patient C001/P03 whose AST levels was outside the normal value throughout the study period. Patient C003/P01 had an initially low value of alanine aminotransferase (ALT) and this remained low till the end of the study. The others had attained normal ALT levels by the end of the study,

even though patients C001/P02 and C002/P01 had levels which were initially outside the reference range at baseline.

Table 4.3 Clinical Chemistry Results of Subjects

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

5.1 DISCUSSION

The introduction of antiretroviral therapy has led to the improvement of quality of life of HIV- infected patients. The main aim of antiretroviral treatment is to suppress viral replication to the lowest possible level whilst enabling the immune system to recover to a certain degree. This study sought to determine how HIV infected patients undergoing herbal treatment would respond to these treatments by particularly looking at how their immune system is improved.

The sample size may appear too small for a work of this nature, but we think since this is a study meant to lead to the selection of possible HIV efficacious herbal drugs for in depth studies, it is quite significant. Especially as already stated, herbal medicine in the country and other places are poorly regulated and not well researched and can sometimes worsen the condition of some patients therefore it would not be advisable to use many study subjects.

5.2 BIOCHEMICAL AND HAEMATOLOGICAL PROFILES

Test results were assessed based on patients' values relative to the normal reference ranges. It was realised that few of the patients had readings at point of initiation of therapy not completely normal but these became normal by the end of the study. On the whole most of the values were within reference range both before and at the end of the study. Just a few of the values which were normal at baseline showed slight changes outside the reference range at the end of the study though these were not indicative enough. Again few of the values at the point initiation that were outside the

normal range at the start of the study remained so throughout the study period. These inferences therefore, suggest that there were no biochemical or haematological related toxicities that could be associated with the intake of the herbal drugs in this study.

5.3 CYTOKINE LEVELS

According to Breen (2002), changes in cytokine levels in HIV- infected patients can affect the immune system and can directly impact the course of HIV disease by enhancing or suppressing HIV replication. In our study it was found that, both IFN- γ and IL- 10 existed throughout the course of the study and none showed significant dominance over the other. This does not correlate with an earlier study that suggests that a shift in the balance of production of Th1 and Th2 cytokines was a major contributor to HIV disease progression (Clerici & Shearer, 1993, 1994). It was also observed that there was no correlation between cytokine kinetics and changes in the $CD4^+$ T cell counts or the plasma viral load.

5.4 $CD4^+$ T CELL AND VIRAL LOAD LEVELS

Observations made by previous studies, indicated that immune deficiencies in people living with HIV and AIDS is the depletion in $CD4^+$ T cell numbers and a high turnover of HIV RNA copies (Saag *et al.,* 1996,).This led to the use of both $CD4^+$ T cell numbers and HIV RNA copies (viral load) to assess the efficacy or otherwise of any HIV/AIDS drug (Fahey *et. al.*, 1990). The observation made in the study was that patients C001/P02 and C001/P03 had a remarkable reduction in viral load and a considerable increase in $CD4^+$ T cell numbers throughout the study period and this can be attributed to their response towards the drug they were taking. However,

patients C002/P01and C003/P01 could not respond to their treatment regimens as indicated by the evidence of both the virological and the immunological data obtained from this study. Therefore, the herbal drug MJ GOLDONI can be said to be potentially antiretroviral considering the effects it has had in the patients who have taken it. However, no conclusion can be drawn on the antiretroviral properties of Misparon OA Unity Mixtures and Amansan Boafo on patients C002/P01and C003/P01 respectively. According to the herbalists of these patients, their medications might not have been properly adhered to, even though they always came for their blood samples to be taken. Improper adherence is known to lead to the emergence of resistant strains and influence viral replication (United States Department of Health and Human Services, 2008). Hence, in view of this we cannot conclude on the antiretroviral nature of Misparon OA Unity Mixtures and Amansan Boafo.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In the treatment of HIV and AIDS, immune reconstitution or restoration is a very important issue. For an antiretroviral treatment to be successful, it is essential that the immunological and virological responses of subjects on treatment be improved. Regarding the herbal drugs Misparon OA Unity mixtures and Amansa Boafo, there were no observed improvements in the immunological and virological status of subjects. It can be said in conclusion that the MJ GOLDONI is efficacious against HIV/AIDS due to the results of its effects on patients who took them. This is indicated by the improvement recorded in the $CD4^+$ T cell counts and reduction in HIV RNA copies of subjects who were on this product.

6.2 RECOMMENDATIONS

Among persons living with HIV and AIDS, there is a widespread use of herbal remedies and some patients will continue to access and adhere to herbal products because they believe these will manage HIV/AIDS infection in them. This should be of major concern to policy makers and health professionals to educate the populace and traditional healers on judicious use of herbal product which may help improve the herbal product aspect of alternate medicine.

Herbal products especially those purported to be efficacious in the treatment of HIV/AIDS should be well researched into and incorporated into the mainstream healthcare system for persons to have easy access to these products.

Finally with regard to the herbal drugs used in this study, we think that the effectiveness of MJ GOLDONI in the management of HIV/AIDS can be addressed only with a longer period of evaluation using a larger sample size for trials. Concerning Misparon OA Unity mixtures and Amansa Boafo, there is not enough justification to question their efficacy but rather, it will be recommended that another study on these herbal preparations with a different set of subjects be carried out.

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APPENDICES

APPENDIX I

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

HERBALIST:

1. Name of treatment

center…….

2. Type of Herbal Center 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 center?

…….

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 center 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 center convinced and interested to participate in it? Yes No

28. If necessary will you give out your drugs 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 …….……..

APENDIX 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, the I will reserve the right to take any action against the administrators of this study.

Signature: ……. Date: ……

Witness: ……...

PARICIPANTS' 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 CENTER……. 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) Christian (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

…….....