CIRCULATING LEVELS OF INTERFERON-GAMMA AND INTERLEUKIN-10 IN HIV-INFECTED PATIENTS UNDERGOING HERBAL THERAPY

by

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ABSTRACT

This study evaluated the expression of IL-10 and IFN-γ in HIV-infected patients undergoing treatment with different herbal drugs. Four (4) HIV-infected patients were enrolled as study subjects and were placed on herbal therapies for an evaluation period of 6 months. Levels of IFN-γ and IL-10, $CD4^+$ T-cell numbers, and plasma viral load levels were measured in the patients before initiation of the therapy and then monthly throughout the study period. Two (2) patients responded successfully to their treatments suggesting the potential potency of the herbal drug they had taken. However, the 2 remaining patients showed evidence of virological and immunological treatment failure. We also found that, both IFN-γ and IL-10 existed in all patients without any marked dominance of one cytokine over the other. These data suggest that HIV infection stimulates the production of both type 1 and type 2 cytokines but does not induce a polarized type 1 or 2 state. Also while IFN-γ and IL-10 may be involved in the pathogenesis of HIV, their profiles in this study are not clear to be used as an index of HIV treatment.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

The standard therapy for the management of HIV/AIDS worldwide and in Ghana is the use of combination treatment, known as the Highly Active Anti-Retroviral Therapy (HAART). Such regimens are usually composed of at least three drugs, currently selected from Nucleoside Reverse Transciptase Inhibitors (NRTIs), Non-nucleoside Reverse Transciptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs) (Montaner *et al*., 1998; Hirschel and Opravil, 1999). However, herbal medicine is also increasingly gaining wide spread recognition in the management of HIV/AIDS. In Africa, for instance, traditional herbal medicine is sometimes used as primary treatment for HIV/AIDS and for HIV-related problems including dermatological disorders, nausea, depression, insomnia, and weakness (Babb *et al*., 2004; Homsy *et al*., 2004; Peters, 2004). However some herbal and traditional medicines are not well-researched, poorly regulated, may contain adulterated products, and may produce adverse effects (Peters, 2004; Ernst, 2002; Morris, 2002). 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). Despite a paucity of evidence on effectiveness, and the possibility of harm, the Ministries of Health of several African nations currently promote traditional herbal medicine for the treatment of HIV and associated symptoms (Morris, 2002; SADC, 2002). The need to monitor the progress of treatment of patients undergoing herbal therapy is therefore imperative to ensure that their already deteriorating conditions are not worsened by the drugs.

The response of HIV/AIDS patients to therapy can be assessed by measuring their $CD4^+$ T-lymphocyte levels and quantifying the amount of viral RNA in the blood (viral load) (Mellors *et al*., 1997). These two markers highly correlate with the clinical progression of HIV disease and are widely used by clinicians in monitoring HIV/AIDS disease progression and response of patients to therapy (Dar and Singh, 1999; Dwyer *et al*., 1997; Fahey *et al*., 1990). HIV infection also stimulates the production of several cytokines some of which seem highly correlated with HIV/AIDS disease and can be used as markers of HIV/AIDS disease progression and assessment of antiretroviral therapy (Tateyama *et al.*, 1994).

A network of cytokines regulates the growth and function of the cells of the immune system. T cells possess a dominant role in this network since they are the main source of many cytokines. The patterns of cytokines produced by T-helper (Th) cells have led to the differentiation of Th cells into two different subsets, Th1 and Th2. Th1 cells produce cytokines such as interleukin (IL)-2, tumor necrosis factor (TNF)-β and interferon (IFN)-γ that promote cell-mediated immunity, while Th2 cells produce cytokines such as IL-4, IL-5, IL-6, IL-10 and IL-13 that promote antibody-mediated immunity (Mosmann *et al*., 1986).

A hypothesis has been introduced suggesting that a shift in the balance of production of Th1 versus Th2 cytokines is a major contributor to HIV disease progression. The hypothesis suggests that early in HIV infection, a vigorous cell-mediated immune response, facilitated by Th1 cells, effectively controls the amount of HIV in the body. However, with time, the predominant cytokine response shifts 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 (Clerici and Shearer, 1994). Other investigators however have either found no change in the cytokine levels or in some cases the opposite result (Fakoya *et al.*, 1997; Graziosi *et al.*, 1994).

Many of the reports supporting the idea of a Th1 to Th2 shift contributing to HIV disease were based on observations of cytokine production following *in* vivo stimulation of peripheral blood mononuclear cells, which are composed of both $CD4^+$ Th and $CD8^+$ T cytotoxic cells, as well as B cells, natural killer cells and monocytes (Breen, 2002). Such observations made under *in vitro* conditions may not directly correspond with *in vivo* cytokine status of cells. In addition, many studies focused on HIV/AIDS patients not on therapy or at best on orthodox therapy or HAART. However, the levels of some cytokines following herbal therapy of HIV/AIDS patients may provide further insight into the Th1/Th2 hypothesis, as well as HIV pathogenesis and herbal therapy in HIV-infected patients.

Among the cytokines, IFN- γ, the hallmark cytokine of Th1 cells, and IL-10, an important Th2 cytokine, have been found to be critical in the pathogenesis of HIV/AIDS. In particular, the balance between the pro-inflammatory cytokines IL-1, IL-6, and TNF-α which up-regulate HIV expression, and IL-10, which can act both as an anti-inflammatory cytokine and a B-cell stimulatory factor, may play an important role in the progression to AIDS (Breen, 2002). Also, IFN-γ expression is very critical in HIV pathogenesis and has been found to inhibit the replication of HIV-1 in latently infected

cells (Lilen *et al*., 2002), while suppressing HIV-induced invasiveness of monocytes (Dhawan *et al*., 1995). Furthermore, IFN-γ and IL-10 are easily detectable in peripheral circulation, and hence are two of the most commonly measured cytokines (Banks, 2000). The study was therefore undertaken to determine the levels of IFN-γ and IL-10 in HIV/AIDS patients undergoing herbal therapy.

1.2 PROBLEM STATEMENT

The use of herbal medicinal products in treating diseases has become widespread and the antiviral and antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri *et al*., 1999; Saxena and Sharma, 1999). Herbal preparations may alter immune function and have had an amazing array of immunomodulatory effects attributed to them. In both mouse and *in vitro* studies, some herbal medicines reportedly inhibit cytokine secretion and the proliferation of T cells (Borchers *et al*., 2000; Ohtake *et al*., 2005). Moreover, T cells play a central role in the regulation of the immune response and are a source of cytokines.

T helper cells secrete cytokines that play an important role in establishing and maximizing the capabilities of the immune system (Mosmann *et al*., 1986). Some of these cytokines have been involved at multiple levels in the pathogenesis of several viral infections including HIV/AIDS (Fauci, 1993, 1996; Poli, 1999). Most of these results regarding cytokine activity, however, have been achieved under *in vitro* condition which may not correspond to *in vivo* conditions. These results, therefore, highlight the need for *in vivo* investigations and circumspection when utilizing herbal drugs and underscore the

need for clinical studies in humans. Meanwhile, the use of traditional medicine is widespread among those living with HIV infection. Hence the ability of herbal medicines to enhance virological and immunological responses in HIV/AIDS patients taking herbal medicines needs to be evaluated.

1.3 JUSTIFICATION

In spite of the efficacy of herbal medicines in the treatment of many diseases, their use as cure for HIV infection has been questionable. While HIV/AIDS is known to be an incurable disease, some herbalists claim that their herbal remedies can completely cure HIV/AIDS. The study was, therefore, undertaken to determine the response of HIV/AIDS patients to herbal preparations by identifying the immunological and virological parameters that are altered in these HIV/AIDS patients taking herbal preparations.

1.4 AIMS

The aim of this investigation therefore is to examine the levels of IFN-γ (produced by Th1 cells) and IL-10 (produced by Th2 cells) to determine whether the levels of these cytokines are related to patients' response to anti-HIV/AIDS herbal therapy.

1.5 SPECIFIC OBJECTIVES

- To determine patients' response to anti-HIV/AIDS herbal therapy.

- To determine the role of IFN-γ and IL-10 in patients' response to anti-HIV/AIDS herbal therapy.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TREATMENT OF HIV/AIDS

The introduction of highly active antiretroviral therapy (HAART) into clinical practice in 1996 has dramatically changed the development of HIV-related diseases (Bonfanti, 1999; Shafer and Vuitton, 1999; Vella and Palmisano, 2000). Although they cannot cure HIV infection and AIDS, the antiretrovirals can reduce morbidity and mortality, prolong lives, and improve the quality of life of many people living with HIV/AIDS (WHO 2002). However, HAART sometimes has a limited response in patients, includes a complicated dosage regimen, and is associated with some drug toxicities. The therapeutic options of treating HIV/AIDS are currently still limited, and alternative approaches are therefore needed. Because of the chronicity and impact of HIV-related diseases on quality of life and the possibility of severe complications and death, patients with HIV/AIDS are likely to seek alternative and complementary therapies (Ozsoy and Ernst, 1999), particularly in areas of the world where HAART is not available or difficult to get.

Herbal medicines in HIV treatment, among the most popular of alternative therapeutic modalities, are defined as products derived from plants or parts of plants used for the treatment of HIV/AIDS. In China and South Africa herbal medicines are used as primary treatments (Liu *et al*., 2009). Also in Ghana, there is widespread use of herbs by HIV/AIDS patients for potential cure or symptom treatment (Selby, 2009).

Herbal medicines might have the potential to reduce symptoms, reduce viral load, and increase CD4+ cells for HIV-infected individuals and AIDS patients (Burack *et al*., 1996; Liu *et al*., 2000; Lu, 1993). On the other hand, there is increasing number of reports in the medical literature about toxicity and other adverse events from some herbal products (Ishizaki *et al*., 1996; Melchart *et al*., 1999), as well as possible herb-drug interactions (Izzo *et al*., 2001). Accordingly, further research and systematic evaluation are necessary.

2.2 MARKERS ASSOCIATED WITH PROGRESSION OF HIV INFECTION

Initial infection with human immunodeficiency virus (HIV) is followed by an asymptomatic period of variable duration characterized by low or absent virus replication, stable or slowly decreasing numbers of $CD4^+$ T-helper cells, and qualitative defects in T-cell function (El-Sadr *et al*., 1987). The pathogenesis of HIV infection involves dynamic interactions between the virus and the host immune system which result in immune activation throughout the course of infection. The degree of activation of the immune system can be monitored by measuring the serum levels of a variety of molecules such as 02-microglobulin and neopterin as well as other serum and cellular markers that correlate with clinical progression of HIV disease (Fahey *et al*., 1990; Hoffman *et al*., 1990; Melmed *et al*., 1989). Because of the varied timing of the development of clinical AIDS following seroconversion for any particular individual, the use of nonclinical disease markers has become critically important to patient management (Dar and Singh, 1999).

These markers for HIV infection have been studied in two disease phases of HIV infection: early (immunocompetent) phase and late (immunodeficient) phase as has been shown in tables 2.1 and 2.2 respectively.

Table 2.1: Some markers found in the early (immunocompetent) disease phase of HIV infection

Type of Marker (Activation)	**Mechanism (Host-dependent)**
Cellular Markers	HLA-DR+ IL-2R+ T Cells
Soluble Markers	ß -M 2 neopterin sIL-2R sCD4 sCD8
Antibody Production	anti-gp120 anti-p24 IgA

(Tsoukas and Bernard, 1994)

Table 2.2: Some markers found in late (immunodeficient) disease phase of HIV infection

Type of Marker (Immune Dysfunction)	**Mechanism (Virus-dependent)**
Cellular Depletion	$CD4^+$ T Cells
Cytokine Depletion	IFN IL-2
Antibody Depletion	anti-p24 anti-gp120

(Tsoukas and Bernard, 1994)

Recently, data on the high viral turnover in HIV infection at all stages of the disease and the value of viral load measurements in antiretroviral drug trials have led to the use of markers that directly measure viral load or other viral characteristics in clinical practice (Saag *et al*., 1996; Volberding, 1996). Measurement of levels of HIV RNA over time has been of great value in delineating the relationship between levels of virus and rates of disease progression, the rate of viral turnover, the relationship between immune system activation and viral replication, and the time to development of antiretroviral drug resistant. (Dar and Singh, 1999; Dwyer *et al*., 1997).

Viral load determination is instrumental in several aspects of the clinical management of HIV infection. First, in early infection at the set point, it serves to assess the likely course the infection will take. Based on the risk of progression, which depends on the viral load, appropriate treatment decisions can be made. The short term efficacy

of a specific antiretroviral treatment can then be assessed by measuring the reduction in virus concentrations achieved within the first 2 to 4 weeks after treatment initiation. Long-term efficacy is likely when virus levels continuously decrease below the level of detection and remain undetectable by the most sensitive assays (Saag *et al*., 1996).

In addition a number of non-HIV specific cellular markers, have been used for staging, monitoring progression of HIV infection and assessing response to therapy but the most commonly used cellular marker is the CD4 lymphocyte count (Fahey *et al*., 1990). Though CD4 cell count is widely used by clinicians, it is a crude predictor of progression. A single abnormal result is not usually a sufficient reason to introduce or change treatment as there are many physiological variables which may affect the count, including the time of day the sample is collected, concurrent infections, and recent exercise (Melone *et al*., 1990).

Higher HIV RNA levels correlate with lower baseline $CD4^+$ T-cell counts, a more rapid decline in $CD4^+$ T-cell counts, and 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. Maintenance of <10,000 copies/mL of plasma HIV RNA in early HIV infection is associated with a decreased risk of progression to AIDS. However, in patients with more advanced disease, a low RNA count does not protect from progression; up to 30% of patients with <10,000 copies/mL progressed (Coombs *et al*., 1996; Saag *et al*., 1996; Welles *et al*., 1996; O'Brien *et al*., 1997)

2.3 CYTOKINES AND HIV PATHOGENESIS

Cytokines, the peptide hormones which control the homeostasis of the immune system and also play a fundamental role in inflammatory and immune mediated reactions, have been involved at multiple levels in the pathogenesis of the acquired immune deficiency syndrome (AIDS). 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 T lymphocytic and mononuclear phagocytic lineage. Activated mononuclear cells, particularly B cells which are in a state of chronic activation 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).

HIV is now known to use not only the CD4 receptor but also the G protein-coupled chemokine receptor family to gain access to various cell types (Clapham and Weiss, 1997). The CC chemokines, macrophage inflammatory protein (MIP)-1α, MIP-1β and RANTES have been identified as the primary mediators of soluble $CD8^+$ T-cell-derived HIV inhibitory activity (Cocchi *et* al., 1995). These chemokines are natural ligands for CCR5, which is the principal co-receptor for macrophage tropic strains of HIV-1 and therefore compete with the virus for binding to CCR5. Also, HIV strains that characteristically utilize CXCR4 (X4 HIV) as a primary co-receptor are inhibited by stroma-derived factor (SDF)-1, the ligand of CXCR4. This can be demonstrated *in vitro*, but the role of endogenous SDF-1 in X4 HIV replication *in vivo* has not been well established (Bleul *et al*., 1996; Oberlin *et al*., 1996).

Furthermore, since cell activation includes HIV expression, virus production is also under the influence of a variety of cytokines that regulate the immune system. Low levels of cytokines are continually secreted even when the immune system appears quiescent and are increased in response to antigen. HIV replicates more efficiently in activated cells, and virus levels consistently increase when the immune systems of HIV-infected persons are activated, e.g., by opportunistic pathogens or by immunization with antigens, such as influenza or tetanus toxoid. Such immune activation is characterized by the cellular activation and expression of the proinflammatory cytokines TNF-α, IL-1β, and IL-6. These agents strongly promote HIV replication. Other cytokines, such as transforming growth factor β (TGF-β), IL-10, and IL-16, downregulate virus replication, in part by downregulating the expression of proinflammatory cytokines (Fauci, 1996). However, since these chemokines may also induce signal transduction, their effects are not restricted to inhibition of viral replication. For example RANTES may block entry of M-tropic virus into $CD4^+$ T cells by interaction with CCR5 but induce activation of the cells by CCR5-mediated signaling, thus increasing CXCR4-mediated infection of the cell by T-tropic viruses (Weissman *et al*., 1997).

Also IFN-γ, a proactive lymphokine with many functions including macrophage activation, is elevated progressively with more advanced disease. IL-2 levels cannot be measured accurately in most serum samples, but IL-2 is known to stimulate IL-2 receptor (IL-2R) production, and elevated serum IL-2R levels are presumed to reflect increased IL-2 production in lymphoid tissues. Regulatory cytokines, such as IL-4, have not been found to be elevated in plasma. It should be remembered that these plasma and serum cytokine levels do not simply reflect the production of cytokines but are also

modified by removal by cell-bound receptors, neutralization by soluble receptors, metabolism, and excretion (Fahey, 1998). Cytokine gene expression occurs during activation and can be evaluated by the measurement of cytokine-specific mRNA in cells of the immune system. Directional changes have been documented, including increases in TNF-α, IL-6, and IFN-γ mRNA, decreased IL-2 mRNA, and approximately normal IL-4 mRNA levels in PBMC (Breen *et al*., 1997; Fan *et al*., 1993; Salazar-Gonzalez *et al*., 1997).

2.4 DIFFERENTIATION OF $CD4^+$ T CELL POPULATIONS

The differentiation of $CD4^+$ T-cell populations into two distinct groups or subsets, Th1 and Th2, is predicated on the patterns of cytokines secreted by the T cells. These distinct T-cell subpopulations determine which types of immune response will be mounted in response to an antigen (Mosmann *et al*., 1986). Th1 cells secrete IFN-γ, TNF-β, and IL-2 whereas Th2 cells secrete cytokines such as IL-4, IL-5, IL-6 and IL-10 (Mosmann and Coffman, 1989). *In vivo* studies have demonstrated that different types of pathogens can elicit discrete responses by either of these Th types.

Cytokines produced by Th1 cells (IFN-γ, TNF-β, and IL-2) lead to macrophage activation, enhanced antigen presentation, and increased Fc receptor expression and superoxide production. These biological activities result in effective killing of intracellular and extracellular pathogens and in the development of long-term immunity to these microorganisms. Th1 cells also seem to be important in mediating delayed-type hypersentivity reactions, including granuloma formation (Mosmann and Coffman, 1989; Skapenko and Schulze-Koops, 2007). Th2 cells on the other hand, produce IL-4, which

stimulates IgE and IgG antibody production, IL-5 (an eosinophil-activating factor), IL-10, and IL-13, which together with IL-4 inhibit macrophage functions. Th2 cells are important in modulating humoral responses to antigens, and are also important in coordinating allergic responses to antigens. These Th2 responses are of vital importance in the immune response to infection with metazoan parasites (Mosmann and Coffman, 1989; Romagnani, 1995).

2.4.1 Interferon Gamma (IFN – γ)

Interferon gamma (IFN-γ) is the hallmark cytokine of Th1 cells. NK cells and CD8+ cytotoxic T cells also produce IFN-γ. Also known as immune interferon, this interferon was originally called macrophage-activating factor (Nathan *et al*., 1983). IFN-γ has antiviral, immunoregulatory, and anti-tumour properties (Schroder *et al*., 2004). Its multiple actions include induction of MHC classes 1 and 2, macrophage activation and increased expression of lymphocyte and endothelial cell adhesion molecules. With TNF-α as the trigger it also increases nitric oxide production by neutrophils, macrophages and endothelium. It alters transcription in up to 30 genes producing a variety of physiological and cellular responses. Amongst the effects are:

- Increasing antigen presentation of macrophages.
- Activating and increasing lysosome activity in macrophages
- Suppressing Th2 cell activity.
- Causing normal cells to increase expression of class I MHC molecules
- Promoting adhesion and binding required for leukocyte migration
- Promoting NK cell activity

In addition, IFN-γ produced by T and NK cells in response to IL-12 acts as a powerful positive feedback mechanism on the phagocytic cells producing IL-12, activating them and enhancing their ability to produce many proinflammatory cytokines, including IL-12 itself (Watford *et al*., 2003).

Moreover, Interferon gamma (IFN-γ) 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. HIV-infected monocytes form highly invasive network on basement membrane matrix and secrete high levels of 92-kd metalloproteinase (MMP-9), an enzyme that degrades basement membrane proteins. However HIV-infected monocytes treated with interferon-gamma 1 day prior to infection with HIV, showed significant reduction in the levels of MMP-9. Thus IFN-γ inhibits HIV-induced invasiveness of monocytes (Dhawan *et al*., 1995). Also, Reuben (2002) 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 (Sarol *et al*., 2002).

2.4.2 Interleukin (IL)–10

Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine mainly produced by the Th2 subset of CD4+ helper cells. However, it is also produced by some activated B cells, some Th1 cells (in humans), activated macrophages, and some nonhematopoietic sources (e.g.,

keratinocytes, colon carcinoma, melanoma cells) (Fiorentino *et al*., 1989; Benjamin *et al*.,1992; Burdin *et al*., 1993; Moore *et al*., 1993; Mosmann, 1994).

Although IL-10 is known to have many different roles in the immune system, its two major activities include inhibition of cytokine production by macrophages and inhibition of the accessory functions of macrophages during T cell activation (Fiorentino *et al*., 1991). The effects of these actions cause IL-10 to play mainly an anti-inflammatory role in the immune system. It down-regulates the expression of Th1 cytokines, MHC class II antigens, and co stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. This cytokine can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway. Knockout studies in mice have suggested the function of this cytokine as an essential immunoregulator in the intestinal tract (Moore *et al*., 2001).

Since its description a little more than a decade ago, IL-10 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 neutralization of endogenous IL-10 may improve defective antigen-specific T cell function in HIV-infected patients (Benjamin *et al*., 1992; Clerici and 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 a*l., 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; Graziozi *et al*., 1994) there are other reports (Emilie *et al*., 1992, 1997) 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 development of AIDS-associated non-Hodgkin's lymphoma (AIDS-lymphoma) (Breen, 2002).

While IL-10 may be beneficial when acting as an anti-inflammatory cytokine that suppresses the production of HIV, IL-10 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 when examining rates of progression 5 years or more after HIV infection. Compared with the general population, persons with AIDS have a > 70-fold increase in the risk of developing AIDS-lymphoma which is thought to be a result of widespread, non-specific B-cell hyperactivity associated with HIV infection (Knowles, 1997). Other findings suggest a pathogenic role for IL-10 in HIV infection (Stylianou *et al*., 1999).

2.4.3 Dynamics of Cytokine Expression in HIV Infected Primary $CD4^+$ T Cells

The interaction between cytokines and HIV expression is multifaceted. Approaches to evaluate the relationship between cytokines and HIV have relied on:

- evaluating the impact of a particular cytokine on HIV replication in vitro, (Steffens *et al.*, 2002; Al-Harthi *et al.*, 1998; Rabbi *et al.*, 1997; Foli *et al.*, 1995)
- comparing the cytokine expression profile of HIV-infected and uninfected donors (Clerici *et al.*, 1993; Imami *et al.*, 2002) and
- measuring cytokine production from *in vitro* infected cells (Gendelman *et al.*, 1990; Kawamura *et al.*, 2003; Ott *et al.*, 1997; Rabbi *et al.*, 1997)

Earlier studies describing the cytokine profile in purified peripheral blood mononuclear cells (PBMCs) from HIV patients have led to discordant findings. Some suggest that HIV-1 infection leads to a shift from type 1 (IL-2, IFN- γ) to type 2 (IL-4, IL-10) cytokines with HIV disease progression (Clerici *et al.*, 1993). An imbalance in the Th1-type and Th2-type responses contributes to the immune dysregulation associated with HIV infection, and that resistance to HIV infection and/or progression to AIDS is dependent on a Th1→Th2 dominance. This hypothesis is based on findings that:

- progression to AIDS is characterized by loss of IL-2 and IFN-γ production concomitant with increases in IL-4 and IL-10; and
- many seronegative, HIV-exposed individuals generate strong Th1-type responses to HIV antigens.

It was proposed that early in HIV infection, a vigorous cell-mediated immunity 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 (Clerici and Shearer, 1994).

By contrast, others have not been able to document such a type 1 to type 2 cytokine shift with disease progression, (Romagnani and Maggi, 1994; Estaquier *et al*., 1995). Again others did not observe a difference between HIV infectivity of Th1 and Th2 subsets (Mikovits *et al*., 1998).

CHAPTER THREE

3.0 METHODOLOGY

3.1 HERBAL THERAPY CENTERS

For our study, traditional herbal medicine practitioners from six herbal therapy centers as listed below were contacted.

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

These herbal centers are known to treat patients with various diseases and also claim to treat HIV/AIDS patients as well. Information about the herbal centers, including their location, number of years of practice, diseases treated and information about their HIV/AIDS herbal preparations, was collected after herbalists from these centers had completed a designed form and questionnaire (Appendix 1) regarding the profiles of their respective herbal centers and preparations.

The herbal centers were to provide their own patients who would be willing to take their herbal preparations. However only the three herbal centers listed below could recruit patients for the study and hence these centers and their anti-HIV/AIDS herbal preparations were selected for the study.

1. M and Jay Health Management Consult
2. Obeng Memorial Herbal Clinic
3. Amansan Boafo Herbal Center

The selected herbal centers were then assigned codes (C001-C003) by which they were identified. Thus:

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

The profile of the three centers is as summarized in table 3.1.

Table 3.1 Profile of Herbal Centers

Centre profile	Centre 1	Centre 2	Centre 3
Name	M and Jay Health Management Consult	Obeng Memorial Herbal clinic	Amansan 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	1999	1992	1994
Other diseases treated by the HIV/AIDS herbal preparation	None	Broad spectrum of infections and cancers	Asthma, whites, chronic diarrhea, breast cancer, eye disease

Table 3.1 continued

Centre profile	Centre 1	Centre 2	Centre 3
Name	M and Jay Health Management Consult	Obeng Memorial Herbal clinic	Amansan Boafo
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 and/or when opportunistic diseases decline	When symptoms are vanishing
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

3.2 HERBAL PREPARATIONS

The herbal drugs evaluated in the study were drugs produced by the three selected herbal centers and were purported to cure HIV/AIDS. Descriptions of the herbal drugs used in the study have been shown in table 3.2.

Table 3.2 Profile of Herbal Preparations

Drug Profile	**Center 1**	**Center 2**	**Center 3**
Name of herbal preparation	MJ GOLDONI	Misparon OA, Unity mixtures	Amansan Boafo
How potency of herbal preparation 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 parts	Plants roots and stem bark	Nyamedua nhini, mahogany, etc which part

Table 3.2 continued

Drug Profile	Center 1	Center 2	Center 3
Name of herbal preparation	MJ GOLDONI	Misparon OA, Unity mixtures	Amansan Boafo
Maintenance	Stabilized with sodium benzoate, keep in cool place		Mixed sodium benzyl
Shelf-life	One and half years	Three years	Three years
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

3.3 STUDY SUBJECTS

Six study subjects, comprising three males and three females, who were all HIV positive and had never had any antiretroviral treatment before, were enrolled into the study as study subjects by the three selected herbal centers. Laboratory tests were performed to confirm their HIV status. The subjects were then requested to complete a designed form (Appendix 2) consisting of a questionnaire, an informed consent form, and a data sheet about their backgrounds. From this, the baseline characteristics of subjects (table 3.3) were acquired and they were given codes by which they were identified.

Table 3.3 Profile of Study Subjects

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 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

Before the inception of the study, the clinical and physical conditions of subjects were assessed by a qualified medical doctor upon consultation after which a sample of venous blood was taken for blood sampling. The subjects were also to be put on their respective herbal medicinal drugs by their herbalists. They were made aware of the need to adhere to the scheduled regimen of the herbal therapy and give sufficient time for regular visits to us throughout the study period. Drug administration was then started and continued throughout the study period. Assessment of subjects' clinical and physical conditions, and blood sampling were continued monthly over a six-month period.

Moreover, during every data collection period, the HIV status of subjects was checked to ascertain whether there could be any change.

3.4 LABORATORY TESTS

To determine patients' response to anti-HIV/AIDS herbal therapy, the following laboratory tests were carried out and results recorded at base line, before initiation of the therapy and thereafter monthly over a six-month period:

1. HIV Antibody tests
2. Hematology
3. $CD4^{+}$T cell counts
4. Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Cytokine Levels
5. Chemistry
6. Measurement of Plasma Viral Load

For these tests, venous blood samples were collected from each patient. 6mL of each sample 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, Kremsmuenster, Austria). Some of the EDTA anticoagulated blood was assayed for HIV antibody by Determine HIV-1/2 Antibody test (ABBOT, Minato-ku, Tokyo, Japan), for hematology parameters, and for CD4+ lymphocyte numbers. From the remaining EDTA anticoagulated blood, plasma was isolated by centrifugation. The plasma was pipetted into 1000µL pyrogen-free plain tubes leaving the blood-cell sediment which was discarded. At least four aliquots of cell-free plasma were collected from each sample on each occasion and the cell-free plasma

was stored at -70°C until assayed for HIV viral load quantity. Samples were frozen and thawed only once.

Also from the blood samples in the plain tubes, serum was isolated by centrifugation after the samples had been allowed to clot (within one hour). Some of the serum was assayed for HIV antibody by OraQuick HIV-1/2 Antibody test (OraSure Technologies, Bethlehem, PA, USA) and for clinical chemistry parameters, while the rest was aliquoted into 1000µL pyrogen-free plain tubes. At least four aliquots of serum were collected from each sample and were stored at -80°C until assayed for IFN-γ and IL-10 levels. Samples were frozen and thawed only once.

3.4.1 HIV Antibody tests

HIV seropositivity of subjects was determined by Determine HIV-1/2 Antibody test (ABBOT, Minato-ku, Tokyo, Japan) and was confirmed by OraQuick HIV-1/2 Antibody test (OraSure Technologies, Bethlehem, PA, USA) as follows:

1. **Determine HIV-1/2 Antibody test:** This assay is a rapid *in vitro*, visually read, immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. Determine HIV-1/2 Antibody test has a sensitivity of 100%. 50µl of each serum sample was added to the sample pad of a Determine HIV-1/2 test strip and results were read between 15 and 60 minutes after the sample had migrated across the test strip. For each test, red bars appeared in both the control window (labeled “control”) and the patient window (labeled “patient”) of the test trip. The red bar at the patient window indicated

that antibodies to HIV present in the sample had reacted with HIV antigen immobilized on the test strip, and therefore confirming a reactive test. Also, the red bar at the control window indicated the validity of the test.

2. **OraQuick HIV-1/2 Antibody test:** This assay is a rapid *in vitro*, visually read, qualitative, immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 in human oral fluid, serum, plasma or whole blood. OraQuick HIV-1/2 Antibody test has a sensitivity of 99.3%. Using a micropipette, 5µl of each whole blood sample was pipetted into a vial of developer solution of the test kit and a loop was used to stir the mixture thoroughly. The pad end of the test device was then inserted all the way down into the vial and results were read in 20 to 40 minutes after the sample had migrated across the test strip. For all samples, a red line appeared on the test ("T") area and another on the control ("C") area of the test strip. The red line in the test area indicated that antibodies to HIV present in the sample had reacted with HIV antigen immobilized on the test strip, and therefore confirming a reactive test. Also, the red line in the control area indicated the specimen contained antibodies to HIV and that the test device was functioning properly therefore confirming the validity of the test.

3.4.2 Hematology

For hematological analyses blood in EDTA-containing tube was processed within two hours of handling. Assessment of haemoglobin level, the quantification of blood platelets and of white blood cell numbers, and differential counts were done using the Auto Hematology Analyzer, BC-3000 Plus (MINDRAY, Nanshan, Shenzhen,

China). The BC-3000 (plate 3.1) is an automated hematology analyzer that provides primary white blood cell (WBC) count, all differential information and 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.

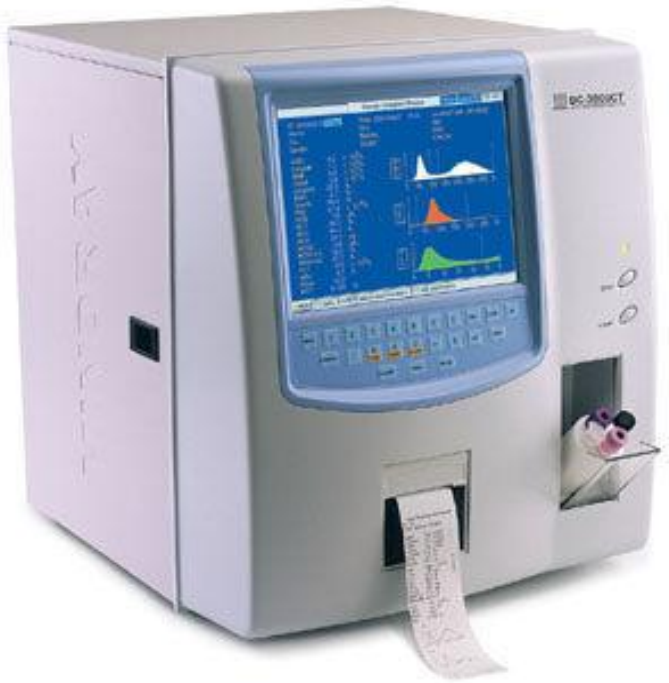

Figure 3.1 BC-3000 Plus

3.4.3 Clinical Chemistry

The biochemical profiles of study subjects were assessed using BT 3000 PLUS (Biotecnica Instruments, Licenza, Rome, Italy), an automatic analyzer for clinical chemistry and immunoturbidity. The BT 3000 PLUS (plate 3.2) is equipped with a software that offers maximum flexibility in the acquisition and performing of tests on serum, plasma and urine. In the procedure, 100µL of serum was analyzed for the liver

enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), and also for total bilirubin, creatinine and Blood Urea Nitrogen (BUN)

Figure 3.2 BT 3000 PLUS

3.4.4 $CD4^{+}$T cell counts

For the enumeration of CD4+ lymphocyte numbers, blood in EDTA-containing tube was processed within four hours of handling. Enumeration of CD4+ lymphocyte numbers in whole blood was assessed using four-color flow cytometric analysis with BD FACSCalibur System (BD Biosciences, San Jose, CA, USA). The FACSCalibur system (plate 3.3) is a benchtop, flow cytometry system that is capable of both analyzing and

sorting cells. Apart from its flow cytometer, the machine is equipped with a FACStation computer and an optional automated sample loader. The whole blood lysis procedure and BD MultiTEST reagent (BD Biosciences, San Jose, CA, USA) were used in the test.

In the test procedure, 20µl of Mutitest Reagent was pipetted into TruCOUNT tubes (BD Biosciences, San Jose, CA, USA) labeled according to the samples. 50µl of each well-mixed whole blood sample was then pipetted into its corresponding tube after which the mixture was agitated gently on a vortex mixer. Following incubation of the tubes at room temperature in the dark for 15 minutes, 450µl of FACS Lysing Solution (BD Biosciences, San Jose, CA, USA) was added to each tube and the mixture was again vortexed. Before usage, 1 part of the 10x concentrate FACS Lysing Solution had been diluted with 10 parts of room temperature deionised water to form 1x concentrate FACS Lysing Solution. After a further incubation of tubes at room temperature in the dark for 15 minutes, they were vortexed thoroughly at low speed to reduce aggregation and then fed into the automated sample loader on the FACSCalibur sysem. Using the appropriate software on the FACStation computer, the samples were run to identify lymphocytes and lymphocyte subsets. The CD4 lymphocyte count of each sample was then recorded from the results obtained.

Figure 3.3 BD FACSCalibur System

3.4.5 Measurement of Plasma Viral Load

The COBAS AMPLICOR HIV-1 MONITOR test version 1.5 (v1.5) (Roche Molecular Systems, Branchburg, NJ, USA) was used to quantify plasma viral load in cell-free plasma. The COBAS AMPLICOR HIV-1 MONITOR v1.5 is an *in vitro* nucleic acid amplification test for the quantification of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma for use on the COBAS AMPLICOR Analyzer (Roche Molecular Systems, Branchburg, NJ, USA). The COBAS AMLICOR Analyzer (plate 3.4) is an instrument that fully automates the amplification and detection steps of the polymerase chain reaction (PCR) process. Quantitation Standard RNA and Master Mix reagent were also included in the Amplicor HIV-1 Monitor test kit.

The test could facilitate the quantification of HIV-1 RNA over the range of 400-750,000 copies/ml and was based on 5 major processes:

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.
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 absorbance of which was measured by the COBAS AMPLICOR Analyzer at a wavelength of 660nm.

The quantitation of HIV-1 viral RNA was performed 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 through the specimen preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target and was amplified together with the HIV-1 target. The COBAS AMPLICOR Analyzer 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.

The COBAS AMPLICOR HIV-1 MONITOR, v1.5 permits simultaneous reverse transcription and PCR amplification of HIV-1 and HIV-1 Quantitation Standard RNA. The Master Mix reagent used in the process contains the primer pair, SK145 and SKCC1B, specific for both HIV-1 RNA and HIV-1 Quantitation Standard RNA and has been developed to yield equivalent quantitation of group M subtypes of HIV -1.

Figure 3.4 COBAS AMPLICOR Analyzer

3.4.6 Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Cytokine Levels

The quantitative determination of IFN-γ and IL-10 levels was done using a commercial enzyme linked immunosorbent assay (ELISA) kit, ELISA Ready-SET-Go! (eBioscience, San Diego, CA, USA). This Human IFN-γ and IL-10 ELISA Ready-SET-Go! Reagent sets contained the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays. The IFN- γ ELISA had a sensitivity of 4pg/mL and a standard curve range of 4-500pg/mL, whereas the IL-10 ELISA had a sensitivity of 2pg/mL and a standard curve range of 2-300pg/mL. Each reagent set consisted of:

1. Capture Antibody (Pre-titrated, purified antibody)
2. Detection Antibody (Pre-titrated, biotin conjugated antibody)
3. Standard (Recombinant cytokine for generating standard curve and calibrating samples)
4. ELISA/ELISPOT Coating Buffer Powder: This was reconstituted to 1L with dionised water and filtered using 0.22µm non-pyrogenic sterile filters.
5. Assay Diluent (5x concentrated): One part of 5x concentrated Assay Diluent was diluted with 4 parts of deionised water to form 1x Assay Diluent and was used throughout the experimental procedure.
6. Detection enzyme (pre-tritrated Aviding HRP)
7. Substrate solution (Tetramethylbenzidine [TMB] Substrate Solution)
8. Certificate of analysis (Lot-specific instructions for dilution of antibodies and standards): Before usage, all antibodies and standards were diluted according to instruction on Certificate of Analysis.

Separate assays were carried out for the quantitative determination of IF-γ and IL-10 levels. Also, all standards and serum samples were assayed in quadruplicates. In the experimental procedure, Fisher 96-well ELISA plates (Fisher Scientific, Pittsburgh, PA, USA) were coated with 100µl /well of capture antibody after which the plates were sealed and incubated overnight at 4°C. The wells were then aspirated and washed manually 5 times with >250µl/well of Wash Buffer of 1x Phosphate Buffered Solution (PBS) containing 0/05% Tween-20. Following the wash step, the wells were blocked with 200ul/well of 1x Assay Diluent and plates were incubated at room temperature for 1hour. After additional washing, standards were diluted and 100µl/well of standards

were added to appropriate wells. 2-fold serial dilutions of the top of standards were performed to make the standard curve after which 100µl/well of samples were added to appropriate wells. The plates were then sealed and incubated at room temperature for 2 hours. This was succeeded by a third wash step followed by the addition of 100µl/well of detection antibody diluted in 1x Assay Diluent after which the plates were sealed and incubated at room temperature for 1hour. Again, the wells were washed and 100µl of Avidin-HRP diluted in 1x Assay Diluent was added to each well. Following incubation of plates at room temperature for 30 minutes, the wells were washed before 100µl of Substrate Solution was added to each well. The plates were then sealed and incubated at room temperature for 15 minutes after which 50µl of Stop Solution of 1M H_3PO_4 was added to each well. Finally, the plates were read at 450nm using an automated microplate ELISA reader, Multiskan EX (Thermo Fisher Scientific, Waltham, MA USA). From the results obtained, a standard curve was run on each assay plate using Microsoft Office Excel 2007 (Microsoft, Redmond, Washington, USA). The concentrations of IF-γ and IL-10 in the samples were calculated by interpolation from their standard curves.

CHAPTER FOUR

4.0 RESULTS

4.1 CHARACTERISTICS OF HERBAL CENTERS

The three herbal centers, M and Jay Health Management Consult, Obeng Memorial Herbal Clinic and Amansan Boafo Herbal Center, that were able to provide patients were selected for the study. All herbal centers are located in the Ashanti region of Ghana and have had considerable number of years of practice. The centers also claim to have treated HIV/AIDS patients before. A detailed description of each herbal center is as follows.

M AND JAY HEALTH MANAGEMENT CONSULT

M and Jay Health Management Consult, is run by an organization and is situated at Mampong in the Ashanti Region of Ghana. They have been practicing for 8 years and treat a number of diseases including HIV/AIDS, diabetic ulcers, buruli ulcer, hepatitis and liver cirrhosis. Treatment of HIV/AIDS at this center began in the year 1999 with a preparation called MJ GOLDONI, which specifically treats HIV/AIDS and no other diseases. At the start of the study, 720 HIV patients had been treated at this center. Also at this center, HIV treatment lasts a minimum of 6 months and response of patients to treatment is assessed by several laboratory markers including CD4 cell count, Liver Function Test (LFT) and Renal Function Test (RFT).

OBENG MEMORIAL HERBAL CLINIC

Obeng Memorial Herbal Clinic is a Kumasi-based clinic owned by an individual. This herbal center, which deals with a considerable number of diseases including

HIV/AIDS, has had 12 years' experience in the field of herbal therapy. HIV/AIDS treatment began at this center in the year 1992 with a preparation known as Misparon OA Unity Mixtures, which is also potent against a broad spectrum of infections and cancers. At the start of the study, 26 HIV patients had been treated at this center. Also, patients' response to treatment, which lasts for 6 to 12 months, is evaluated using laboratory markers or by monitoring declines in opportunistic infections.

AMANSAN BOAFO HERBAL CENTER

Amansan Boafo Herbal Center is also Kumasi-based and is owned by an individual. Having been practicing herbal medicine for 14 years, Amansan Boafo is recognized for the treatment of HIV/AIDS and other diseases. HIV/AIDS treatment began at this center in the year 1994 with a herbal preparation known as Amansan Boafo, that also treats Asthma, whites, chronic diarrhea, breast cancer and eye diseases. Over 100 HIV patients had been treated at this center before the inception of the study. HIV/AIDS patients qualify for therapy at this center when they present symptoms like weight loss and persistent fever, while treatment is evaluated by monitoring declines in these symptoms. At this center, HIV/AIDS treatment lasts for 6 to 8 months.

4.2 CHARACTERISTICS OF HERBAL PREPARATIONS

The purported antiretroviral preparations under evaluation were produced by the three selected herbal centers.

MJ GOLDONI

MJ GOLDONI produced by M and Jay Health Management Consult is made from the parts of some medicinal plants by the water extraction method. This herbal preparation is stabilized with sodium benzoate and then kept in a cool place for maintenance. It has a shelf life of one and half years and is administered orally, three times daily. Among the herbal preparations used in our study, MJ GOLDONI is the only one with known side effects which included suppression of appetite, transient acute weakness and hepatotoxicitiy if misused.

MISPARON OA UNITY MIXTURES

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

AMANSAN BOAFO

Amansan Boafo is also a liquid mixture produced by Amansan Boafo Herbal Center from the parts of some plants by the water extraction method. Amansan Boafo has shelf life of three years and is maintained by mixing with sodium benzyl. During the course of treatment, one tablespoonful of this herbal preparation is taken orally, three times daily. Amansan Boafo like Misparon OA, Unity Mixtures, has no known side effects.

4.3 CHARACTERISTICS OF STUDY SUBJECTS

All patients used for the study had tested positive for HIV and were naive for any antiretroviral drugs prior to enrollment. Of our six study subjects, four (C001/P02, C001/P03, C002/P01, C003/P01) successfully completed the program. Patient C001/P02 was a 44-year-old female divorcee who was a Christian and lived in an urban community. She was recruited by M and Jay Health Management Consult just like patient C001/P03. C001/P03 was a 45-year-old male divorcee who was also a Christian and lived in a semi-urban community. Patient C002/P01, recruited by Obeng Memorial Herbal Clinic, was a 40-year-old married man who lived in a semi-urban community and was a Christian. The only patient of Islamic faith among our subjects was C003/P01, drafted into the study by Amansan Boafo Herbal Clinic. He was 34 years of age, lived in a rural settlement and had never been married. Also, with the exception of patient C001/P03, who had been educated up to the Secondary School level, the other subjects who successfully completed the study had all been educated only up to the primary school level. In addition all four of our subjects were low income earners very much in vindication of the relationship between poverty and HIV/AIDS in Ghana.

4.4 BLOOD SAMPLING

4.4.1 Hematological Profiles

As shown in table 4.1, at baseline only the hemoglobin level and hematocrit value of patient C002/P01 were within the reference range. Also, all patients had their platelet counts falling within the reference range and with the exception of patient C001/P02, whose WBC count was 0.1 short of the lowest reference value, all of them had their WBC counts within the reference range. Again, assessment of the differential

white cell count saw the percentage of granulocytes (gran) in patients C002/P01 falling within the normal range while the other patients recorded comparatively low values. The percentage of lymphocytes (lym) in patients C002/P01 and C003/P01 was normal as against the high percentages attained by the remaining patients. Patient C001/P03 also had a normal percentage of mid cells (mid) - consisting of monocytes, eosinophils, basophils, blasts and other precursor white cells - with the remaining patients recording high percentages.

At the end of the study period, the hemoglobin levels and hematocrit counts of patients C001/P02 and C001/P03 became normal whereas that of patients C002/P01 and C003/P01 fell outside the normal range. Also no patient recorded a normal WBC count apart from C003/P01 who attained a WBC count of $5.5x10^9$/L. Patient C003/P01 also was the only patient who attained an abnormal platelet count of $384*10^9$/L. Regarding differential white cell counts, patients C002/P01 and C003/P01 attained normal granulocyte and lymphocyte counts while patients C001/P02 and C001/P03 had comparatively low granulocyte percentages and high lymphocyte percentages. However, only patient C001/P03 had a normal percentage of mid cells of 7.4% while the other patients had high values.

Table 4.1 Hematology Results 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 4.1 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

4.4.2 Biochemical Profiles

Table 4.2 below shows the clinical chemistry results of the study subjects. At baseline before initiation of therapy, all patients had normal total bilirubin values and these values still remained normal at the end of the study period. Also, patients C001/P02, C001/P03 and C002/P01 had normal Blood Urea Nitrogen (BUN) levels which remained within the reference range at the end of the study period. However patient C001/P03 had an abnormal BUN level of 25.0mg/mL which continued to remain outside the reference range at the end of the study period. The creatinine level of patient C001/P02 at baseline was the only normal value among the values attained by the four study subjects and this value remained normal at the end of the study period, while the remaining patients still had their creatinine levels outside the normal range.

With respect to the liver enzymes, only patient C001/P03 had his aspartate aminotransferase (ALT) level falling outside the reference range at baseline, and there it remained at the end of the study period. Also, while patients C001/P02 and C002/P01 had normal values at the beginning, the level of gamma-glutamyl transferase (GGT) in patient C001/P03 was unconventionally high and remained high in the end. Patient C003/P01 also had had a high GGT value at the beginning but managed to attain a normal value, while the level of this enzyme in patients C001/P02 and C002/P01 fell outside the normal range at the end of the study period. Secondly, all patients maintained normal aspartatate aminotransferase (AST) levels throughout the study period, except patient C001/P03 who had abnormal values both at baseline and at the end of the study. Finally, patients C001/P02, C001/P03 and C002/P01 attained normal alanine aminotransferase (ALT) levels at the end of the study period, even though patients

C001/P02 and C002/P01 had initially had levels which had been outside the reference range at baseline. The initially low level of ALT in patient C003/P01 however, still remained low but slightly outside the normal range at the end of the study period.

Table 4.2 Clinical Chemistry Results of Study Subjects

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

Table 4.2 continued

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

4.4.3 $CD4^+$T cell counts

$CD4^+$T cell counts quantified over the treatment period in the study subjects have been shown in figure 4.1 below. Patient C001/P02 attained increases in $CD4^+$T cell numbers over the treatment period and then suffered a slight decline in the last week of treatment as has been shown in figure 4.1A. Similarly, after having a very low baseline count of 5cells/ml, patient C001/P03 experienced an increase in $CD4^+$T cell numbers in the 8^{th} week and then sustained an increase in the 16^{th} after which $CD4^+$T cell numbers declined marginally to 253cells/ml in the last week of study period as shown in figure 4.1B. In contradiction to these results, those obtained for C002/P01 with $CD4^+$T cell numbers declining throughout the study period as shown in figure 4.1C. Also, $CD4^+$T cell count results for patient C003/P01 fluctuated throughout the study period as shown in figure 4.1D.

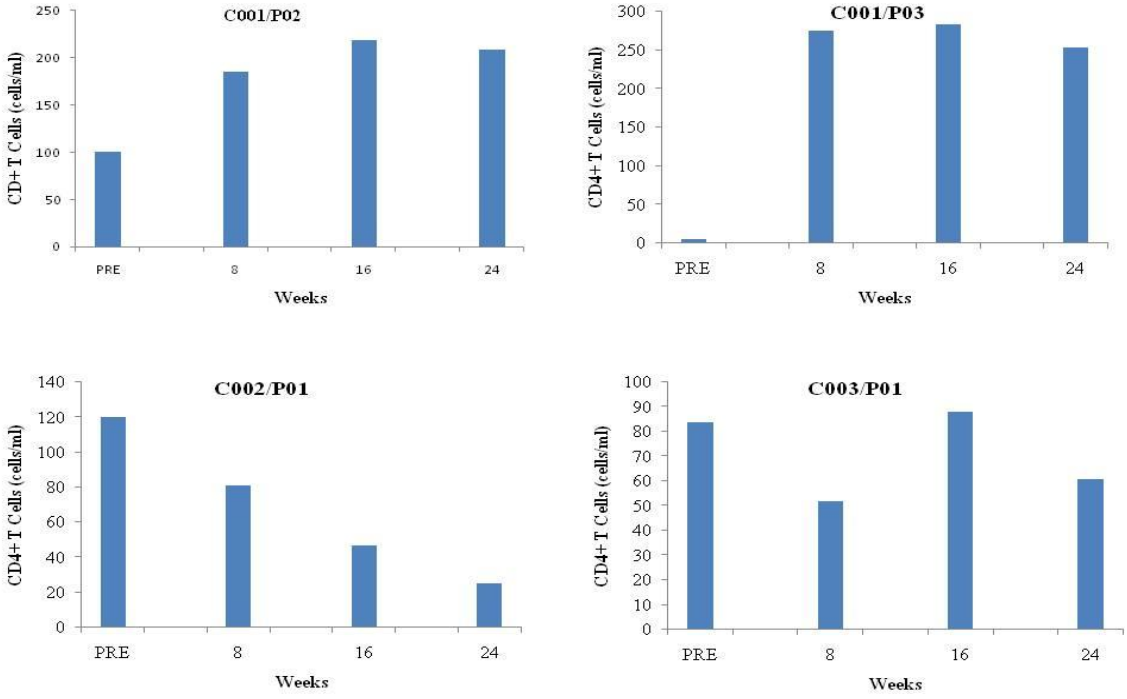

Figure 4.1 $CD4^+$T Cell Counts of Study Subjects

4.4.4` Plasma viral load

The management of the disease in patients C001/P02 was associated with a considerable decline in plasma viral load from a baseline level of 940000copies/mL to 1790copies/mL in the 8th week, and then finally falling to undetectable levels throughout the study period as shown in figure 4.2A. This provided evidence for a relative decline in viral replication. Similarly, a decline in viral replication occurred in patient C001/P03 (figure 4.2B) as viral load decreased from a baseline value of 849000copies/mL to 21900copies/ml, and then declined to undetectable levels afterwards.

However viral load regarding patient C002/P01 remained high throughout the treatment period, declining marginally in the 16th week and then elevating again in the last week of treatment as shown in figure 4.2C. Also, viral load results for patient C003/P01 (figure 4.2D) were quite irregular and fluctuating, beginning with a substantially high viral load turn out of 3130000copies/mL and then ending with 1220000copies/mL at the end of the study period.

Table 4.3 Plasma Viral Load Levels of Study Subjects

	Treatment Weeks	C001/P02	C001/P03	C002/P01	C003/P01
Plasma Viral Load (copies/mL)	PRE	9.4×10^5	8.5×10^5	3.2×10^5	$3. \times 10^6$
	8	< 400	1130	3.1×10^5	1.1×10^6
	16	< 400	< 400	2.5×10^5	1.9×10^6
	24	< 400	< 400	8.0×10^5	1.2×10^6

4.4.5 Cytokine levels

Serum levels of IL-10 and IFN-γ in the four HIV-infected patients are shown in figure 4.2.

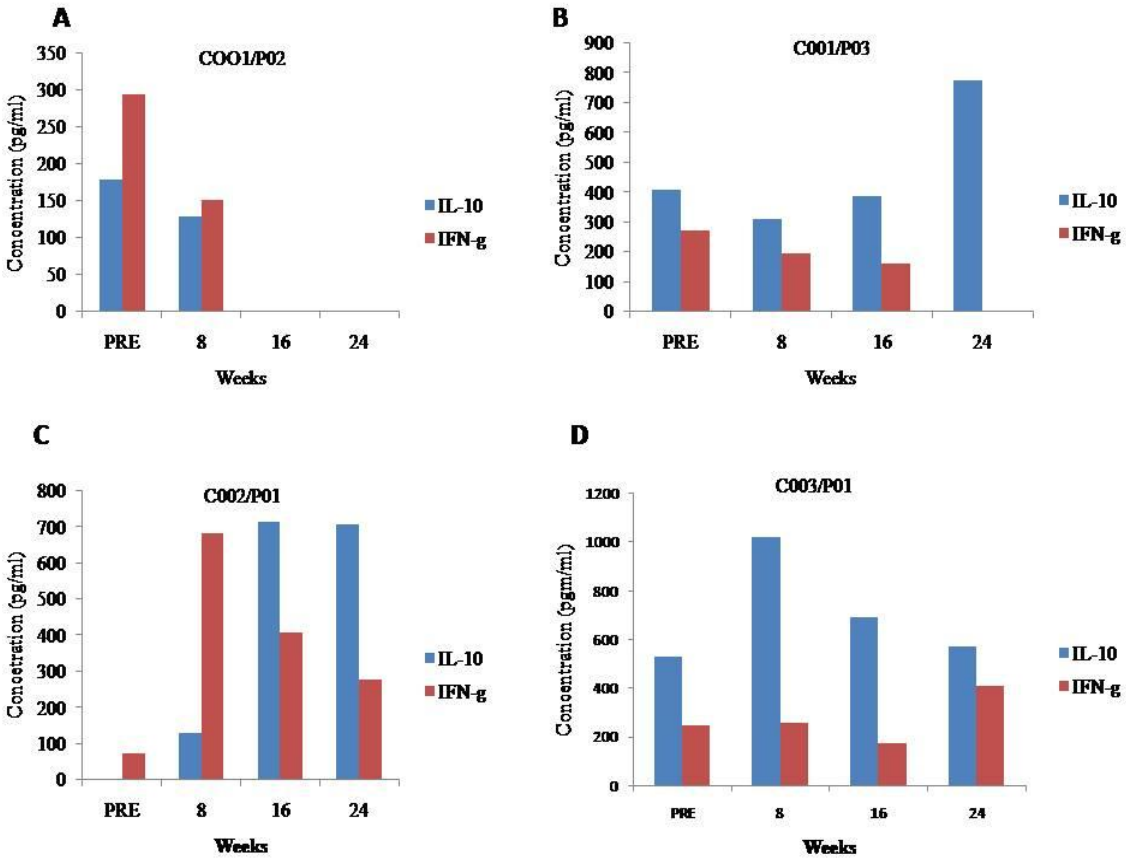

Figure 4.2 IL-10 and IFN-γ Levels in Study Subjects

The baseline concentration of IL-10 and IFN-γ in patient C001/P02 were 179pg/mL and 296 pg/mL respectively. However a decline in the concentrations of the two cytokines to levels below the detection limit of the assay was recorded from the 16th week onwards as shown in figure 4.3A. This occurred after IL-10 concentration had decreased to 129pg/mL and IFN-γ concentration had also declined 15pg/mL.

Also, with a baseline IFN-γ concentration of 273pg/mL, patient C001/P03 sustained decreases in IFN-γ level throughout the study period until the level of that cytokine finally fell below the detection limit of the assay in the last week of the study period as shown in figure 4.3B. In the same patient however, contradictory results were observed regarding IL-10 levels with the concentration of that cytokine dropping in the 8^{th} week and then increasing gradually in the course of the study period with the highest concentration of 775pg/ml recorded in the last week of treatment.

From figure 4.4C, treatment of the disease in patient C002/P01 was marked by an increase in the concentration of IL-10 in the 8^{th} week after the assay had failed to detect it at baseline before inception of the therapy. This was followed by a considerable increase to 715pg/ml in the 16^{th} week and this level suffered a marginal decline in the last week of treatment. Moreover, beginning with a low IFN-γ concentration of 73pg/ml before the initiation of the therapy, a considerable increase to 684pg/ml was recorded in the 8^{th} week of treatment. The level of IFN-γ then declined throughout the study period.

Assessment of cytokine levels in patient C003/P01 as shown in figure 4.4D revealed the following: A low IL-10 concentration was recorded at baseline before initiation of the therapy. This was followed by a significant elevation to 1025pg/ml in the 8^{th} week of treatment after which it declined throughout the study period. Furthermore, there was a decline in the level of IFN-γ in the 16^{th} week after the cytokine had elevated narrowly above its baseline concentration of 250pg/ml. The concentration then increased to 410pg/ml in the last week of treatment.

CHAPTER FIVE

5.0 DISCUSSION

In this study we evaluated the expression of IL-10 and IFN-γ in HIV-infected patients undergoing treatment with different herbal preparations with the aim of determining the role these cytokines play in the pathogenesis of HIV infection. We also measured $CD4^+$ T cell numbers and plasma viral load levels in these patients in order to assess patients' response to the herbal preparations.

5.1 CHALLENGES OF THE STUDY

We had initially expected each of the 6 contacted herbal centers to provide 3 patients for the study. However, 3 herbal centers withdrew from the program on grounds of patient recruitment problems. Even of the 3 remaining herbal centers, only center C001 could provide 3 patients, with centers C002 and C003, providing 2 and 1 respectively. In the end, only 4 out of these 6 subjects could successfully complete the study. Nonetheless, this did not in any way undermine our study as the ultimate aim was to select herbal drugs with very high potential as HIV/AIDS therapeutic for in-depth evaluation.

5.2 $CD4^+$ T CELL COUNTS AND PLASMA VIRAL LOAD LEVELS IN PATIENTS

It has been observed that the major immune deficit in persons with AIDS is the marked depletion in $CD4^+$ T cell numbers coupled with high HIV RNA (viral load) levels. Patients' response to a specific antiretroviral treatment can be assessed by measuring the reduction in virus concentrations and an increase $CD4^+$ T cell numbers

(Dar and Singh, 1999; Dwyer *et al*., 1997; Fahey *et al*., 1990). In this study, we observed that patients C001/P02 and C001/P03 had persistent suppression of viral load and sustained increase in $CD4^+$ T cells attributable to their good response to treatment. On the contrary, patients C002/P01 and C003/P01 showed evidence of virological and immunological treatment failure. This can be inferred from patient C002/P01's elevating viral load levels and declining $CD4^+$ T cell counts, and patient C003/P01's high viral load levels and fluctuating $CD4^+$ T cell counts. This further attests the potential of the herbal preparation MJ GOLDONI taken by patients C001/P02 and C001/P03 as having antiretroviral properties compared with the preparations, Misparon OA Unity mixtures and Amansan Boafo taken by patients C002/P01 and C003/P01 respectively. However, we cannot discredit the potency of Misparon OA, Unity mixtures and Amansan Boafo due to some reasons.

Firstly, patient C002/P01, who took Misparon OA Unity mixtures, was a very intractable person. He had suffered an emotional breakdown and became depressed on account of his unexpected affliction and possibly because of his wife (patient C002/P02) moving away from him. His problem was so severe that he had on several occasions threatened to take his own life. This situation concurs with a new study which has found that depression can severely worsen HIV treatment in patients (Horberg *et al*., 2008). Horberg *et al*. (2008) showed that depression could play a significant role in discouraging a patient from being loyal to antiretroviral therapy and clinical measures. We therefore believe that our patient's depressed state may have precluded his adhering properly to the treatment process.

Secondly, we understood from the herbalist of patient C003/P01 that this patient used to take the drug at some odd times and sometimes never. The patient's discouraging response to the treatment could therefore be attributed to his non-compliance to the treatment schedule involving the herbal preparation Amansan Boafo. Compliance to therapy affects how well anti-HIV medications decrease a patient's viral load since when a medication dose is skipped the virus has the opportunity to reproduce more rapidly. Also, improper adherence to HIV treatment may lead to the development of HIV strains that are resistant to the medication being taken (United States Department of Health and Human Services, 2008).

5.3 HEMATOLOGICAL AND BIOCHEMICAL PROFILES OF PATIENTS

Some herbal and traditional medicines are not well-researched, poorly regulated, may contain adulterated products, and may produce adverse effects (Peters *et al.*, 2004; Ernst, 2002; Morris, 2002) thereby aggravating the conditions of the patients who are using them. Considering these concerns, we assessed the hematological and biochemical profiles of our patients in order to ascertain the possible toxicity of the herbal preparations used in our study.

We measured of the following clinical chemistry parameters:

1. Creatinine to investigate disorders of renal function and monitor uremia
2. Bilirubin to diagnose and monitor jaundice
3. Liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) to investigate liver disease.

We also measured the following hematology parameters:

1. Hemoglobin (Hb) to detect anemia

2. Hematocrit to evaluate the mean cell hemoglobin concentration and the mean cell volume used in the investigation of anemia
3. White Blood Cell (WBC) count to investigate unexplained fevers and leucopenia
4. Platelet count to investigate abnormal skin and mucosal bleeding, cytotoxicity and thrombocytopenia
5. Differential white cell counts to diagnose an illness affecting the immune system and other diseases that affect the white blood cells.

The interpretation of test results was based on assessment of patients' values relative to the normal reference ranges. We found that some of the values were within the normal reference ranges before and at the end of the study, indicating normal pathological conditions. Also, a few values had been abnormal at baseline but ended up becoming normal. In a few instances however, the values had initially been normal at baseline but were deflected just outside the normal reference ranges at the end of the study period. The slight deviation of those values from the reference ranges was not considered as significant. According to Elston (2006), laboratory results that are slightly or moderately outside the normal reference ranges should be given little attention as they are of marginal clinical significance. Finally, we also found that values that had been utterly abnormal at the start of the study unsurprisingly still remained far from the normal values at the end of the study, although some of those values were improved slightly. These abnormal results we believed indicated pathological defects that had afflicted our study subjects before the start of the study, and of which the study herbal drugs could neither curb nor aggravate. Considering these inferences therefore, we are of the view that there were no hematological or biochemical toxicities that could be ascribed to the ingestion of the herbal preparations under evaluation.

5.4 CYTOKINE LEVELS IN PATIENTS

Immune response can be broadly categorized into cellular and humoral response. The production of interferon (IFN)-γ leads to a Th-1 type cellular response, while the production of interleukin (IL)-10 leads to a Th-2 type humoral immunity (Mosmann and Coffman, 1989). Changes in the cytokine levels in HIV-infected persons can affect the function of the immune system and have the potential to directly impact the course of HIV disease by enhancing or suppressing HIV replication (Breen, 2002). Earlier studies describing cytokine expression in HIV infection suggested that HIV infection leads to a shift from type 1 to type 2 cytokines with HIV disease progression (Clerici *et al*., 1993); although this suggestion has been controverted by other reports (Fakoya *et al.*, 1997; Graziosi *et al.*, 1994). If HIV infection really induces such a shift, then antiretroviral therapy is expected to reverse this trend.

In the present study, we observed that both IFN-γ and IL-10 existed in all patients throughout the course of the study and there was no significant dominance of one cytokine over the other. The information presented in the current study seems to be in agreement with previous studies that HIV infection stimulates the production of both type 1 and type 2 cytokines but does not induce a polarized type 1 or 2 state (Fakoya *et al*., 1997; Graziosi *et al*., 1994). However we believe that these cytokine appeared to be involved in the pathogenecity of HIV infection.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Immune reconstitution is a critical issue in the treatment of HIV infection. Successful response to antiretroviral treatment is associated with improvement in virological and immunological responses with normalization of many markers of immune activation. In this respect, we can conclude that the herbal preparation MJ GOLDONI is potentially efficacious against HIV infection because of the desirable results it has produced in the patients who have taken it.

Furthermore, the results suggest that IFN-γ and IL-10 may be associated with HIV pathogenesis. However, their profiles are not clear enough to establish an index between the levels of these cytokines and the pathogenecity of HIV infection. Again, the expression of both IFN-γ and IL-10 in our study subjects with no distinctive dominance of one cytokine over the other demonstrates that HIV infection induces the expression of both Th1 and Th2 cytokines but does not induce a polarized type 1 or 2 state.

6.2 RECOMMENDATIONS

The widespread use of herbal compounds by people living with HIV/AIDS should be of concern to clinicians and policy makers. Clearly, patients will continue to access traditional healing systems as it is important to local cultural values and beliefs. Therefore, efforts should be made by mainstream health professionals to provide validated information to traditional healers and patients on the judicious use of herbal

remedies. This may reduce harm through failed expectations, pharmacologic adverse events and unnecessary added therapeutic costs. Efforts should also be directed at evaluating the possible benefits of natural products in HIV treatment.

Regarding the herbal preparations used in this study, we think that whether MJ GOLDONI is effective in the management of HIV infection can be adequately addressed only by a larger trial of longer duration. We therefore recommend a large-scale study with a larger sample size to test the efficacy of this herbal preparation. Also, since we do not have enough reason to discredit the efficacy of Misparon OA Unity mixtures and Amansan Boafo, we suggest that another study of these herbal preparations with reliable subjects will help.

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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 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 by 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 my 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, then 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) 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 ……..