KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
SCHOOL OF MEDICAL SCIENCES, CLINICAL MICROBIOLOGY,
KUMASI

# HIV, HBV AND HCV CO-INFECTED PATIENTS' RESPONSE TO THE HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN THE VOLTA REGION OF GHANA

BY

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# DECLARATION

This study was carried out at the ART centres and laboratories of Krachi West District Hospital, Hohoe Municipal Hospital and Volta Regional Hospital under the supervision of Dr Theophilus B. Kwofie

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# DEDICATION

To my beloved, Juliet Anukpui and my family, who were very supportive throughout the course of my study. It is also dedicated to all persons attending ART centres in the Volta Region of Ghana who made this study possible. To God be the glory, great things He has done.

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ALT | - | Alanine Aminotransferase |
| AST | - | Aspartate Aminotransferase |
| ALP | - | Alkaline Phosphatase |
| AIDS | - | Acquired Immune Deficiency Syndrome |
| Anti-HBc | - | Antibodies to Hepatitis B core Antigen |
| Anti-HBs | - | Antibodies to Hepatitis B surface Antigen |
| ART | - | Antiretroviral Therapy |
| ARV | - | Antiretroviral |
| CDC | - | Centers for Disease Control and Prevention |
| EDTA | - | Ethylene Diamine Tetra-Acetic Acid |
| ELISA | - | Enzyme Linked Immunosorbent Assay |
| ESLD | - | End-Stage Liver Disease |
| GHS | - | Ghana Health Service |
| HBeAg | - | Hepatitis B e Antigen |
| HBsAg | - | Hepatitis B surface Antigen |
| HBV | - | Hepatitis B virus |
| HCC | - | Hepatocellular carcinoma |
| HCV | - | Hepatitis C virus |
| HIV | - | Human Immunodeficiency Virus |
| HAART | - | Highly Active Antiretroviral Therapy |
| HSS | - | HIV Sentinel Surveillance |
| IVDU | - | Intravenous Drug Users |
| MoH | - | Ministry of Health |

| | | |
|---|---|---|
| MSM | - | Men who have sex with men |
| NACP | - | National AIDS/STI Control Programme |
| PCR | - | Polymerase Chain Reaction |
| RBV | - | Rivabirin |
| SD | - | Standard Deviation |
| SVR | - | Sustained Virologic Response |
| UNAIDS | - | Joint United Nations Programme on HIV and AIDS |

# ABSTRACT

Human immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C (HCV) share similar routes of transmission and pose serious public health concerns. Viral hepatitis co-infection with HIV is known to impact negatively on treatment response among HIV patients. This study sought to determine the treatment outcome of HAART on mono-infected HIV patients and HIV patients co-infected with viral hepatitis and the prevalence of Hepatitis B and/ or C co-infections among HIV patients in the Volta region of Ghana.

A prospective cohort study design was used in this study. HIV-1 positive patients attending ART centres were recruited for the study from January, 2014 to December, 2015. Two hundred blood samples were collected from the cohort and screened for the presence of hepatitis B (HBsAg) and C (anti-HCV antibody). A total of one hundred and ten study participants comprised of HIV mono-infected (93 participants) and hepatitis co-infected HIV patients (17 participants) were prospectively followed for one year. CD4, body weight, haemoglobin and liver enzymes levels were determined for each participant at baseline, six months and twelve months intervals.

Among the HIV-1 positive individuals, 14 patients were infected with hepatitis B virus (7.5%), 3 with hepatitis C virus (1.5%) and none triply infected. The overall hepatitis-HIV prevalence was 8.5%.

There was significant increase in the mean CD4 count, weight gain and haemoglobin levels among HIV mono-infected patients from baseline through six month to the twelve month of therapy ($p, <0.05$) whereas the co-infected group presented with no significant change in the

aforementioned variables over the same period. There was a negative treatment response among hepatitis co-infected HIV patients as compared to mono-infected counterparts. The findings underscore the importance of screening for hepatitis B and hepatitis C viruses in the HIV infected individuals on therapy.

Keywords: Human Immunodeficiency Virus; Hepatitis B virus; Hepatitis C virus; co-infection.

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND

Triple infections of Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), and Hepatitis C Virus (HCV) are serious public health problems. HIV-HCV and/or HIV-HBV co-infections are reported as common since they share similar routes of transmission. There is a significant decline in the survival rate, with a higher risk of progression to severe liver diseases and hepatotoxicity associated with antiretroviral therapy among HIV-HCV and/or HIV-HBV co-infected patients (Mohammadi *et al*, 2009). In spite of the current reduction in the mortality and morbidity rate from HIV/AIDS due to increased accessibility of Highly Active Antiretroviral Therapy (HAART), hepatic diseases attributable to chronic HBV and HCV infections have become the primary cause of death among HIV positive patients (Mallet *et al*, 2011).

Hepatitis B and C viruses are the two major causes of chronic liver diseases worldwide. The health of many people around the world therefore continues to be severely impacted by these infections which are often asymptomatic (Alberti, 2005). In most developing countries like Ghana, the management and monitoring of viral hepatitis is not fully integrated in anti-retroviral therapy (ART) programmes, despite the fact that some anti-retrovirals are known to be effective against HBV. As such, these infections remain undiagnosed and the affected individuals are ignored (Easterbrook *et al*, 2012). Even though, it is largely recommended that HIV infected patients be tested for hepatitis prior to anti-retroviral therapy (HAART), this is however not the case in Ghana due to logistical constraints and lack of trained laboratory staff

in most of the treatment centres. Limited epidemiological information on the impact of viral hepatitis in HIV patients may underline reasons for many developing countries, including Ghana not incorporating hepatitis services in HIV infected persons. As a result, patients co-infected with HBV and HCV are ignored with regards to when to start therapy, the choice of ARV regimens, and screening for cirrhosis of the liver as well as hepatocellular carcinoma (HCC).

It is estimated worldwide that 10.0% and a third of the people infected with HIV have chronic HBV and HCV infections respectively (Fenton, 2007). HIV, HBV, and HCV co-infections are common in sub-Saharan Africa. In a systematic review to address the extent of the situation in Africa (Barth, 2010), published the mean prevalence rates of HIV/HBV as 15% and HIV/HCV as 7% among HIV-positive people. However, there is a wide variation in the co-infection pattern worldwide. These variations are said to be dependent on the geographical location, the type of exposure involved and the risk groups which may be different among countries and regions of the same country and also the socioeconomic condition of that particular region (Koziel *et al*, 2007; Saha *et al*, 2011). Ghana is not an exception to these phenomena considering her socioeconomic and cultural diversity.

Volta Region is one of the ten administrative regions of Ghana with an over 2.2 million population and shares a geographical border with Togo to the Eastern side with thriving socioeconomic activities. The region is reported having an estimated HIV prevalence rate of 2.2% as against the national rate of 1.3% in 2014 (NACP report, June 2015). There is currently, a complete absence of testing for HBV and HCV among HIV patients in most centres providing ART services in Ghana.

This present study in the Volta Region therefore sought to determine the prevalence and the impact of viral hepatitis on HIV patients and their response to HAART. This will provide findings of clinical importance to be used in the formulation of therapeutic policies to lessen the burden on co-infected HIV patients on treatment. This is imperative since HBV and HCV treatment is very costly, and its associated toxicity which relies on expensive laboratory tests (Easterbrook *et al*, 2012). Such data will also highlight the need for nationwide surveillance programmes and routine laboratory testing for both hepatitis B and C among HIV infected patients.

## 1.2 PROBLEM STATEMENT

HIV/AIDS remains a manageable chronic disease since there is no cure. HIV mono-infected patients respond favorably to the HAART treatment, but when co-infected with viral hepatitis, the response is poor. Thus, HBV and HCV have the potential to complicate the prognosis of HIV co-infected patients. Hepatitis co-infection with HIV increases the progression of HIV/AIDS and hepatotoxicity associated with the intake of antiretrovirals.

In HIV-HBV/HCV co-infected patients, there is a rapid devastating effect on their liver resulting in various abnormalities especially among the lower CD4 group (Romeo, 2000; Thio 2009). Additionally, HIV co-infected patients are unable to recover immunologically (Christian *et al*, 2010).

With an increased use and accessibility of HAART among HIV positive patients in Ghana, co-infection with these viruses could contribute significantly to continuing morbidity and

mortality among this group of patients. Although, this occurrence varies widely from country to country and region to region (Mohammadi, 2009), and despite the public health concerns, very few studies have reported on the impact of HIV and viral hepatitis co-infection in Africa in general and Ghana in particular. Currently in Ghana, routine laboratory detection of HBV and HCV among HIV positive patients is simply non-existing, owing to the unavailability of testing materials at the treatment centres. Moreover, there is no systematic survey on the prevalence of HBV and HCV among HIV positive patients that has been conducted in the Volta Region of Ghana. The extent of the problem therefore remains unclear since there are no published data currently available to evaluate the situation from this part of the country. This study therefore sought to address the problem in the Volta Region of Ghana by determining whether HIV co-infected patients are responding favorably to HAART or otherwise.

## 1.3 JUSTIFICATION

Patients on highly active antiretroviral therapy (HAART) live much longer; but unfortunately, liver diseases are becoming the main cause of death among HIV patients co-infected with hepatitis. Data available confirms the high prevalence of viral hepatitis among HIV patients across Africa (Barth *et al*, 2010).

However, no comprehensive study on HIV, HBV and HCV co-infection among patients attending ART centres and its effect on the highly active antiretroviral therapy had been done particularly in the Volta Region of Ghana. The present work was designed to assess the situation in antiretroviral therapy (ART) centres in the Volta Region of Ghana. Moreover,

there is the absence of routine testing for the presence of hepatitis in the management of HIV/AIDS currently in treatment centres in Ghana. Additionally, most healthcare workers at these centres are unaware of the extent and effect of the problem and therefore are inept in making decisions regarding the choice of ARVs in achieving optimum treatment response among this group of patients. The consequences have been several treatment failures and deaths of patients with their economic burdens.

The result of this study would therefore provide the baseline for future larger studies and would serve as basis for public health education especially among HIV patients for policy planning and health advocacy.

### 1.4 AIM

This study was aimed to evaluate the impact of hepatitis co-infection on HIV-infected individuals' response to HAART in the Volta Region of Ghana.

#### 1.4.1 SPECIFIC OBJECTIVES

- To determine the treatment outcome of HAART on mono-infected HIV patients and HIV patients co-infected with viral hepatitis.
- To determine the prevalence of Hepatitis B and/ or C co-infections among HIV positive patients.

## 1.5 HYPOTHESIS

I hypothesized that co-infection with hepatitis B virus and/or, hepatitis C virus would increase disease progression in HIV patients as compared to patients infected with HIV alone on therapy (HAART).

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV is responsible for causing Acquired Immune Deficiency Syndrome (AIDS). HIV belongs to the genus Lentivirus and family of Retrovirus (Sharp and Hahn, 2011 pp 1-2). The virus has a cylindrical nucleoid in the mature virion. HIV infects CD4 lymphocytes, causing their gradual depletion leading to immunosuppression. Without any form of intervention, it takes on the average 8-10 years for HIV to develop into AIDS.

According to UNAIDS 2013 report, out of the 35 million HIV population worldwide, 71% are from Sub-Saharan Africa.

In Ghana, the first HIV/AIDS was diagnosed in 1986 (UNAIDS AIDS Epidemic Update, 2000) and although the current national HIV prevalence is low (1.3%), the infection is well established within the society. And a total number of 224,488 Ghanaians in 2013 were projected as living with HIV and AIDS (NACP Report, 2014).

HIV-1 and HIV-2 are the two main variants, with HIV-1 being the predominant type found globally and HIV-2 mostly found in West African nations (Brooks *et al*, 2007; de Silva *et al*, 2008). Infection with HIV occurs through contact with infected blood and sexual contact. Other risk factors include unprotected sexual intercourse with different partners and intravenous drug use. Measures to reduce HIV infection include education on HIV prevention

and AIDS, promotion of condom use and effective diagnosis and treatment of other sexually transmitted diseases.

HIV has no cure; however, the condition can be managed by using a Combination Therapy (three or more anti-HIV drugs). This composition of drugs work against HIV infection by reducing the replication of the virus, boost or strengthen the immune system and also help in the treatment of opportunistic infections in the body.

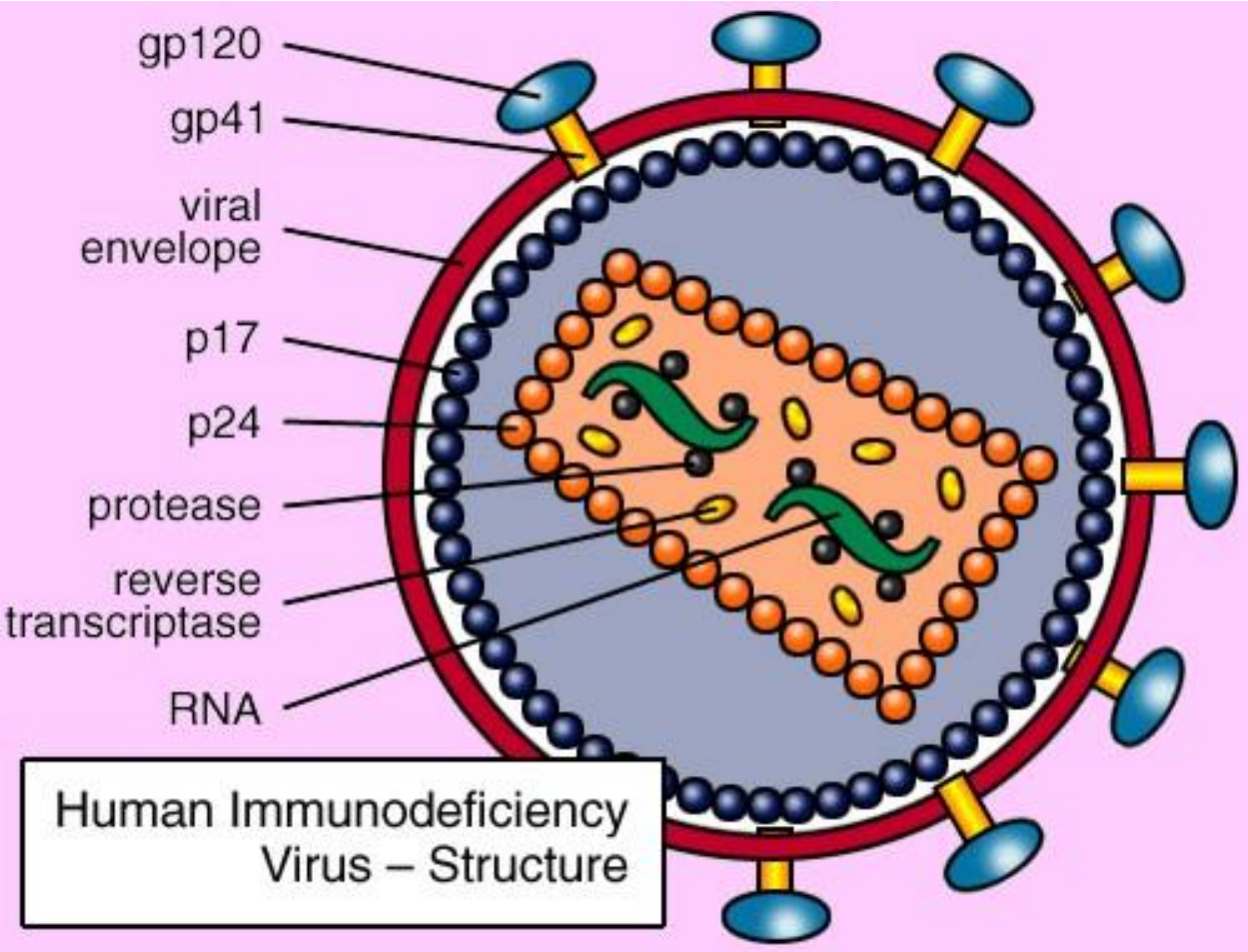


**Figure 1: Structure of HIV virion particle**

### 2.1.2LIFE CYCLE OF HIV

As shown in Fig. 2 below, when the virus attaches itself to the host cell membrane, it then fuses its envelope with the cell membrane resulting in the virus entering the cell. Once inside the cell, there is uncoating and the viral core undergoes a slow dissolution process (Arts and Hazuda, 2012). This process protects the viral RNA but permits access to deoxyribonucleotide triphosphates (dNTPs) which is necessary for reverse transcription and proviral DNA synthesis. Reverse transcriptase (RT) then helps in the conversion of the single-stranded RNA genome into double-stranded DNA (Hughes and Hu, 2011).

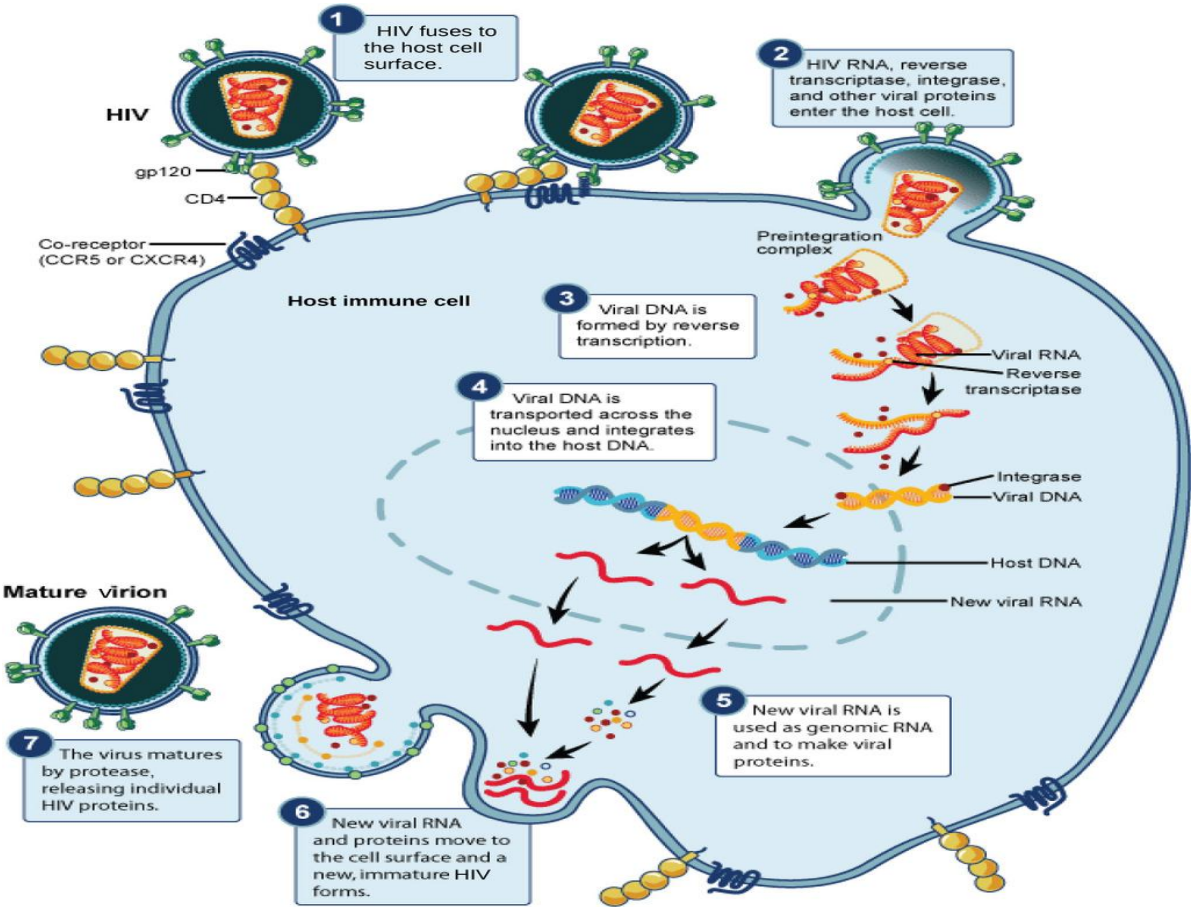


**Figure 2: A schematic diagram of the life cycle of HIV showing the various steps involved in the replication of the virus in a human host (Source: www.boundless.com/anti-HIV drugs)**

### 2.1.3 THE NATURAL COURSE OF HIV INFECTION

Most HIV-infected persons experience an acute HIV illness few weeks after transmission of the virus. There is a flu-like clinical presentation associated with an increase in plasma viremia with fever, maculopapular rash, myalgia, lymphadenopathy and usually last not later than two months. Depending on the individual, other symptoms may be reported (Gurunathan *et al*, 2009).

The level of HIV RNA, which reaches extremely high values as much as 10 million copies/ml (Little *et al*, 1999) soon after primary infection, drops to less than 1% at the time of first HIV antibodies appearance and remains stable at this level for some years. This level is called the viral set point. This determines the rate of disease progression. The higher the levels of HIV RNA copies/ml at viral set point of an infected person the more rapid he/she develops AIDS (O'Brien *et al*, 1996).

CD4 cells usually decrease significantly during acute primary infection and increases a couple of months later to normal range. Normal reference values for CD4 cell counts vary from one laboratory to the other. However, the usual adult range is 435–1600/µl (Hoffmann *et al*, 2012).

In a review conducted by Moir *et al*, revealed a significant increase in CD4 cell counts of most patients on HAART. However, very few patients were seen to have low CD4 cell counts throughout treatment even on virologically suppressive drugs (Reviewed by Moir *et al*, 2011).

HIV infection later progresses to AIDS at a median of 8–10 years after primary infection and without any intervention with HAART, AIDS-defining illnesses eventually result to death of patients after 2- 4 years (Hoffmann *et al*, 2012).

In the era of HAART, the progression of HIV infection to AIDS has been halted. With HAART, there is considerable viral suppression and a massive boost in the CD4 levels and patients experience normal life (Sterne *et al*, 2005).

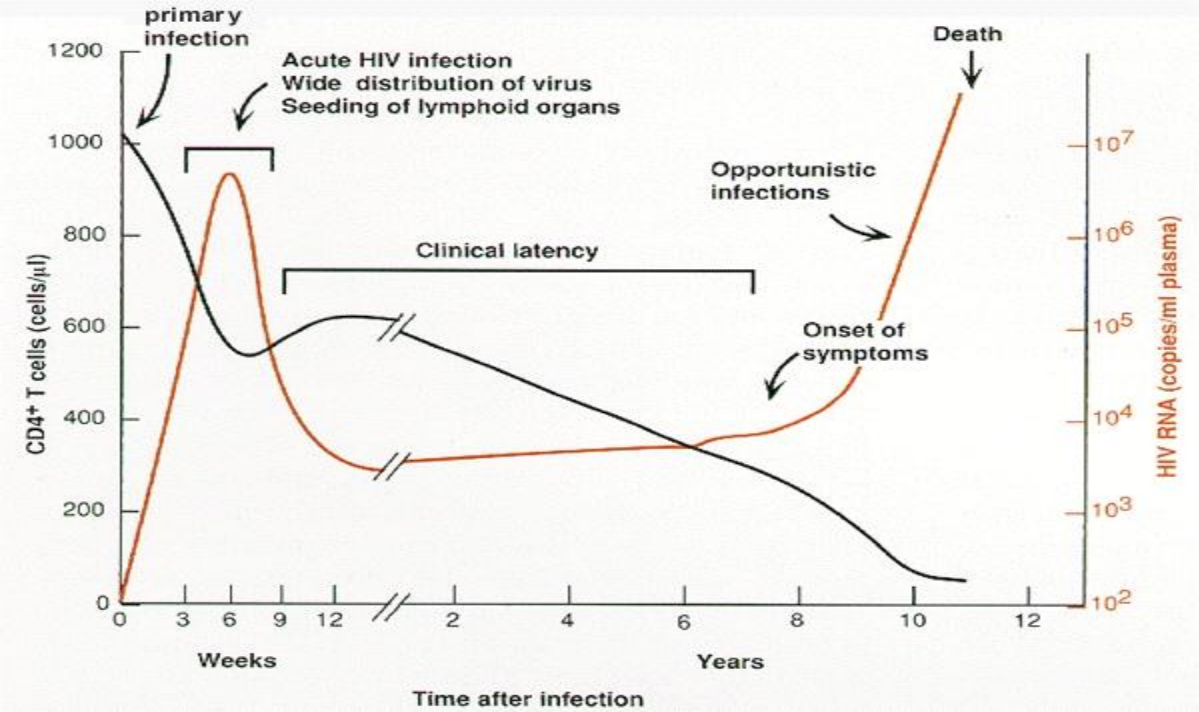


**Figure 3: Natural course of HIV infection (Modified from Pantaleo *et al*. 1993a.)**

### 2.1.4 HIV/AIDS IN GHANA AND VOLTA REGION

Ghana is an English-speaking country in West Africa with a land mass of 238,537 sq. km. (www.ghana.gov.gh, 2015). It shares boarders with three francophone countries: Burkina Faso to the North, La Cote D'Ivoire to the West and Togo to the East. The south of the country is bounded by the Gulf of Guinea. Ghana's population was estimated at 25 million in 2010. Volta region is one of the ten administrative regions of Ghana and has a land mass of 20,570

sq. km with a population of 2.2 million (www.ghana.gov.gh, 2015). The region has been demarcated into 25 administrative districts.

Ghana reported its first HIV case in 1986 (Hishida *et al*, 1994). Since then, the number of cases has steadily increased and HIV infections in the country have been predominantly due to HIV-1 (Brandful *et al*, 1998). HIV-2 and HIV-1/HIV-2 co-infections constitute an approximate 5% of the infections (Bonney *et al*, 2008). The HIV Sentinel Survey (HSS) organized by NACP, which was started in 1992, has consistently, provided epidemiological data on HIV trends in Ghana. An estimated 224,488 persons were infected with the virus in 2013 (NACP, 2014). The predominant HIV-1 subtype is CRF02_AG circulating in Ghana with unique recombinant forms (URFs) (Brandful *et al*, 2012).

The number of people receiving ARVs in Ghana has greatly improved from 2,017 in four treatment sites in 2004, to 65% of total infected people in 2014 (NACP, 2015). The treatment centres have also enjoyed a significant corresponding increase across the country. And these figures are expected to rise even further in the coming years in line with the universal access targets. However, antiretroviral drugs available in Ghana include the NRTIs: stavudine, lamivudine, abacavir, didanosine, tenofovir, zidovudine, emtricitabine; NNRTIs: efavirenz and nevirapine and PIs: lopinavir/ritonavir and nelfinavir (NACP, 2010). With the scale up in ART, there is a need to control the prevalence and impact of viral hepatitis co-infection among HIV patients receiving treatment since this condition could negatively affect their response to therapy. Even though NACP in their treatment guidelines recommends that all HIV positive patients be assessed for hepatitis B infection, there is currently non-compliance to the directive nationwide due to the non-supply of testing materials.

The National ART Guidelines which is based on the World Health Organization's ART guidelines for resource-poor countries recommend the use of two NRTI and one NNRTI in the first-line regimen and two NRTI and one PI in the second-line regimen. The guidelines further recommend the change from first-line to second line when there is evidence of treatment failure (NACP, 2010). However, due to unavailability of viral load, the change in regimen and general response to therapy is determined by clinicians based on clinical symptoms/ assessment and CD4 counts if available.

### 2.1.5 DIAGNOSIS OF HIV INFECTION IN GHANA

Laboratory tests to identify the presence of HIV in infected persons use blood, serum, plasma or mucosal swabs. There are various testing methods (antibody tests, rapid tests, antigen tests, western blot, qualitative PCR) used to detect HIV by viral antibodies, antigens, viral nucleic acids by PCR or grow the virus in culture (Fearon, 2005).

In Ghana, rapid antibody assays such as First Response (Premier Medical Corporation Ltd, India) and OraQuick (OraSure Technologies Inc., USA) are used to screen and subsequently confirm individuals for their HIV status. Rapid HIV tests are EIAs that have all the necessary reagents and produce results in less than 30 minutes. They usually contain antigens made from intact viruses and therefore detect antibodies to most types of HIV (Bulterys *et al*, 2004). These tests are useful to screen people since results are readily available to enhance treatment decisions. Rapid tests are used for the quick determination of HIV status of pregnant women in labour and consequently help to prevent mother-to-child transmission (PMTCT). In the case of an occupational exposure, the index patients sample can also be tested to allow prompt

initiation of ARV prophylaxis to the exposed health care provider or researcher (Bulterys *et al*, 2004). The national algorithm requires that results are declared when the outcome of two rapid assays are consistent. Indeterminate results are clarified on an immunoblot assay such as Inno-lia HIV I/II Score. Inno-lia can also be used to determine the type of HIV infection: HIV-1 or HIV-2. For large scale screening, during surveillance and after a blood donation exercise, ELISAs are used. Fourth generation ELISAs are used for detection of both antigen and antibody (Branson 2007). Qualitative PCR is also used to clarify the status of new-borns to HIV infected mothers and detect HIV infection in occupational exposures to infectious agents.

## 2.2 HEPATITIS B VIRUS (HBV)

HBV is a DNA virus of the family Hepadnaviridae. It has a heterogeneous genome which is classified into 8 genotypes: A, B, C, D, E, F, G and H. Each HBV genotype is known to be geographically segregated (Norder *et al*, 2004). Genotype E (HBV/E) is common in Ghana with an estimated HBV prevalence of 16% in the general population (Geretti *et al*, 2010).

Humans are the only known natural host and replication of HBV occurs only in liver tissue. The infectious virus circulates in high concentrations (about $10^8$virions per ml) in the blood. The inner core of the virus contains hepatitis B core antigen, hepatitis B e antigen (HBeAg), a partially double-stranded 3,200-nucleotide DNA molecule, and DNA polymerase with reverse transcriptase activity. Hepatitis B surface antigen (HBsAg) is found on the surface of the virus and is self-assembling, noninfectious spherical or tubular (Norder *et al*, 1993).

Hepatitis B virus infection presents a serious global health problem. Worldwide, about 4.5 million new HBV infections occur each year, of which a quarter progresses to liver disease (Zanetti, 2008; WHO, 2014). Chronic hepatitis B may progress to severe liver-related diseases.

## 2.3 HEPATITIS C VIRUS (HCV)

HCV is an RNA virus classified in the genus *Hepacivirus* of the family *Flaviviridae* (Shepard *et al*, 2005). The HCV genome displays considerable sequence divergence classified into six genotypes, 1–6. These genotypes and their subtypes display different geographical distributions worldwide (Nakano *et al*, 2012). HCV genotypes 1 & 2 are the two main strain found in Ghana (Markov *et al*, 2009).

Hepatitis C virus infection is a serious public health issue of global concern. HCV causes chronic infection in 80% of exposed people. Chronic HCV infection may progress to severe complications such as hepatocellular carcinoma (HCC) and death. An estimated 3% of the world's population is chronically infected with hepatitis C infection (WHO, 2014).

## 2.4 EPIDEMIOLOGY OF HIV AND VIRAL HEPATITIS COINFECTION

### 2.4.0 INTRODUCTION

According to the World Health Organization (WHO), Hepatitis B virus infection accounts for about 378 million chronic infections, Hepatitis C virus infection for an approximate 150 million, and HIV infection was estimated at 35 million (WHO, 2014). Each of these chronic

infections is known globally to contribute substantial morbidity. Although these viruses share similar routes of transmission, there is a wide variation in prevalence by geographical regions (Koziel *et al*, 2007).

### 2.4.1 HIV AND HBV COINFECTION

Hepatitis B virus (HBV) is more prevalent in human immunodeficiency virus (HIV) infected persons than in the general population. This phenomenon is attributed to the shared risk factors for viral acquisition (Crockett *et al*, 2005). Factors affecting the high rate of chronic HBV include mode of acquisition and age at the time of infection. These factors vary geographically. Injection drug users (IVDU) and men who have sex with men (MSM) have the highest rates of HIV/HBV co-infection. In sub-Saharan Africa, vertical and early childhood exposure is the most common modes of transmission.

Studies (Matthews *et al*, 2003; Thio, 2009) suggest that HIV infection has a negative impact on HBV with an increase in liver mortality and morbidity. HBV was also identified to show more chronicity in HIV infected patients especially with low CD4 counts.

In another study by Bonacini and co (2004), also found liver-related mortality to be 2-3 times higher in HIV/HBV co-infected patients than in HIV mono-infected patients. HIV co-infection with HBV has also been identified to be linked with increases in liver transaminases, which can occur with immune reconstitution inflammatory syndrome (IRIS) owed to HAART, and the development of resistance to HIV/HBV treatment (Lacombe *et al*, 2006).

However, there are conflicting reports from studies with respect to the impact of HBV on the natural history of HIV infection. Some of these studies demonstrated an increased rate of HIV progression to AIDS (Eskild *et al*, 1992; Ockenga *et al*, 1997) while others reported no change in the progression of HIV disease or survival (Law *et al*, 2004).

### 2.4.2 HIV AND HCV COINFECTION

Hepatitis C virus (HCV) is a significant healthcare problem with as many as four million new infections occurring each year (WHO, 2014). HCV may increase the rate of progression to acquired immune-deficiency syndrome (AIDS); impair immune reconstitution and also increase the risk of hepatotoxicity among HIV infected individuals. HCV infection has the tendency to increase the number of complications in persons who are co-infected with HIV (Thomas *et al*, 2011). In the general population, 80% of people exposed to HCV become chronically infected, and at least 30% of the carriers develop chronic liver disease (Sultan *et al*, 2009).

Co-infection with HIV and HCV is common due to shared routes of transmission but with varied prevalence among studies. HIV and HCV are both transmitted through parental, sexual and vertical exposure but differ in the transmission efficiencies of these routes (De Carli *et al*, 2003). For example, in HIV-positive patients with a history of intravenous drug use, the rate of HCV infection is 82-93%, while sexual transmission of HCV is relatively inefficient, with less than 10% risk factor (Mohsen *et al*, 2005).

There is increase in HCV RNA levels in serum and in the liver of co-infected patients after HIV seroconversion which significantly impacts on the life cycle of HCV (Sherman *et al*,

1993). This negative effect is blamed on the interaction between the two viruses. Increased risk for cirrhosis and liver cancer, liver disease progression and a higher mortality rate than the mono-infected are some of the damaging effects of HIV/HBV co-infection (Arnold *et al*, 2006). There is some data on the impact of HCV in HIV co-infected patients that suggest a more rapid progression to AIDS or death, and an impaired immune recovery during therapy, although this is still controversial (Romeo, 2000).

## 2.5 THE IMPACT AND PREVALENCE OF HBV/HCV CO-INFECTION AMONG HIV-1 PATIENTS ON HAART

HAART is noted to have some direct hepatotoxic effects which are aggravated by co-infection with HBV or HCV (Puoti *et al*, 2004). Earlier studies have shown that progression to liver-related diseases (Di Martino *et al*, 2001; Graham *et al*, 2001; Martin-Carbonero *et al*, 2004: Tsuchiya *et al*, 2012) have increased in HIV and viral hepatitis co-infected persons, especially those with low CD4 cell counts.

Prospective data collected by a study group (Weber *et al*, 2006) on the adverse events of antiretrovirals presented liver disease as the second to AIDS-related mortality among patients on therapy. HCV accounted for 66% of these liver-related deaths whereas, 17% were attributable to HBV and 3% traced to HAART.

Currently the impact and prevalence of HIV and viral hepatitis co-infection varies between regions and from country to country (Koziel *et al*, 2007) but most studies have examined incidence in US and Europe, and only a few have studied the situation from Africa.

In developed countries, up to 14% of the HIV population is reported having HBV co-infection and 25 to 50%, co-infected with HCV (Tien, 2005). A study done in Ahvaz - South Iran, put HIV/HBV, HIV/HCV, and HIVHBV/HCV co-infection rates at 44, 74, and 20% respectively (Alavi *et al*, 2007). Moreover, in India, the prevalence rate of HBV in HIV-positive patients was 3.4% while that for HCV was reported as 0% (Mahajan *et al*, 2008). A Swiss HIV cohort (Greub, 2000) reported an accelerated disease progression in both HCV and HBV and an increased risk of antiretroviral drug associated hepatotoxicity among patients co-infection with HIV with slower immunological recovery than in patients with HIV alone. This conclusion was further supported by a meta-analysis report on CD4 counts among similar categories of patients after start of HAART (Miller *et al*, 2005).

In a related work conducted in Australia on 2086 participants by Lincoln and friends (2003), revealed co-infection with HBV and HCV among HIV positive participants to be 4.8% and 10.7% respectively. This same work witnessed a poorer immune response in HIV/HCV co-infected patients. Recently in Cambodia (Belgium), van Griensven and colleagues (2014), reported worse HAART outcomes for HBV and HCV patients co-infected with HIV. They discovered that CD4 recovery was lower in both HBV and HCV co-infection but only statistically significant for HBV (P, 0.001) with a prevalence of 11.0% (HIV/HBV) and 5.3% (HIV/HCV).

In Africa, a study in North-West Ethiopia showed slightly increased levels in liver enzymes and a much lower CD4 counts in viral hepatitis co-infected HIV patients than their mono-infected counterparts. The prevalence for hepatitis among the HIV population was 5.6% (HBV), 5.0% (HCV) and 1.1% (HBV/HCV) respectively (Wondimeneh *et al*, 2013). Another

study in Ethiopia (Taye and Lakew, 2013) revealed a negative recovery in CD4 cells count (poor response to HAART) and a raised AST and ALT enzyme levels in HIV/HCV co-infected individuals on therapy as against HIV mono-infected participants with much favourable response to HAART. Whereas study results of a larger HIV positive population conducted in Nigeria demonstrated the prevalence rates for HBV, HCV, and HBV/HCV co-infections as 11.9%, 4.8%, and 1%, respectively (Otegbayo *et al*, 2008). Taiwo and colleagues (2012), in a similar work in Nigeria with a smaller cohort, observed a higher prevalence for HBV, HCV, and HBV/HCV co-infections as 28%, 14%, and 4%, respectively. Elsewhere in North-Western Nigeria recorded a prevalence rate of 12.3% and 1.6% for hepatitis B and C co-infections with HIV respectively (Hamza *et al*, 2013). In another study from North-Central Nigeria by Forbi and others (2007), revealed that co-infection of HIV and hepatitis viruses (HBV and/or HCV) is on the increase in Nigeria and appears to decrease the CD4 counts of patients who are co-infected especially with triple co-infections of HIV, HBV, and HCV. In a study of 378 HIV positive individuals in Nairobi, Kenya 6% were co-infected with HBV while 1% was co-infected with HCV (Harania *et al*, 2008). Also in another work published (Chiekulie *et al*, 2013) in Orlu, a sub-urban South-Eastern Nigeria, reported a low seroprevalence of HBV (2.2%) and HCV (0.7%) among HIV infected patients. They proceeded to recommend the inclusion of screening for hepatitis in the protocol of all HIV treatment centers even in resource limited settings in order to reduce morbidities and mortalities from liver diseases amongst HIV positive patients.

A systematic review of 60 studies in sub-Saharan Africa by Barth *et al*. in 2010, showed that a considerable number of HIV-infected patients are HBV or HCV co-infected with a mean prevalence rates at 15% and 7%, respectively. Additionally, a meta-analysis was also done

which showed a 40% increased risk for a positive HBsAg and a 60% increased risk for a positive anti-HCV in HIV-infected, as compared with patients without HIV infection (Reviewed by Barth *et al*, 2010).

In Ghana, Geretti and others (2010), reported a prevalence of 16.7% HBV co-infection among HIV patients attending KATH HIV Clinic in Kumasi. In 2012, Sagoe and others also investigated the prevalence and impact of HBV and HCV among antiretroviral treatment naïve HIV patients in a treatment center in Ghana. They reported a prevalence of 13% and 3.6% co-infection for HBsAg and anti-HCV, respectively. The study generally revealed a positive response to therapy among the various groups but witnessed a more severe immune suppression in HBeAg positive co-infected HIV patients as compared to those with anti-HBe.

There is however, conflicting reports from studies with regard to the impact of HBV and HCV on the disease course of HIV infection.

## 2.6 HEPATOTOXICITY IN HIVAND HEPATITIS COINFECTION

Management of HIV using HAART regimen is associated with some adverse reactions such as hepatotoxicity which may be life threatening, despite the remarkable improvement in the survival of patients. Hepatotoxicity can interfere with the effectiveness of HIV therapy and cause an increase in morbidity and mortality (Sulkowski *et al*, 2003).

Protease Inhibitors and non-nucleoside reverse transcriptase inhibitors are known to exert hepatotoxic effect on HIV patients on therapy, but with asymptomatic elevations in transaminase levels and, rarely, liver failure and death (N´u˜nez *et al*, 2005). Often viral

hepatitis patients have elevated liver enzymes which can be worsened with the inclusion of hepatotoxic anti-retrovirals for the treatment of their HIV infection. It has also been reported that co-infection with viral hepatitis increases the possibility for hepatotoxicity of HAART and likelihood of an AIDS related illness, compared to HIV-1 mono-infected patients (Cooper, 2007).

However, several studies have indicated mild hepatotoxicity amongst patients co-infected with hepatitis B or C virus upon initiation of therapy (Heil *et al*, 2010; Kalyesubula *et al*, 2011). Other investigators have also reported a rise in incidence of hepatic injury in patients on HAART with identified hepatotoxic events (Lucien *et al*, 2010; Teklay *et al*, 2013). In Uganda, there is a documented evidence of the absence of hepatotoxicity among patients on HAART (Kalyesubula *et al*, 2011). The above clearly demonstrates the variation in incidence rates of hepatotoxicity during therapy across different populations and therefore the need to determine the situation in our setting.

## 2.7 TRANSMISSION AND PREVENTION OF MULTIPLE INFECTIONS

### 2.7.1 ROUTE OF TRANSMISSION OF VIRAL HEPATITIS

The understanding of the routes of transmission of HIV and viral hepatitis co-infection is crucial since it is related to sociodemographic factors such as the level of health education on prevention in a particular geographical region (Nwokedi *et al*, 2006; Lesi *et al*, 2007). HIV, HBV, and HCV infections share similar route of transmission. Transmission is principally through blood and its products, urine, semen, sweat, saliva and tears, use of contaminated

needles, sexual contact, intravenous drug abuse and vertical transmission (mother to child transmission) (Nwokedi *et al*, 2006; Lesi *et al*, 2007; Gillham, 2010).

### 2.7.2 PREVENTING AND MINIMIZING HBV/HCV CO-INFECTION AMONG HIV POPULATION

It is imperative to strengthen educational programs targeted at reducing risk of hepatitis infection among HIV population. HIV positive individuals should be counseled not to share personal items like sharps, toothbrushes and instruments that may come in contact with an infected person's blood.

Vento and others investigated HIV infected patients in 1998 and recommended that, nonimmune patients should be vaccinated against hepatitis B infection since they could acquire it later in life. And in the case of prevention of hepatitis C infection, the incidence can be reduced through continuous education on transmission patterns.

Blood donor screening, education and infection control practices to be encouraged to reduce the risk of new infections. Also, healthcare providers play a critical role in counseling patients on transmission, risk reduction by abstaining from sexual intercourse, being monogamous, use of condom and being tested for sexually transmitted diseases.

## 2.8 TREATMENT RESPONSE TO HIV AND VIRAL HEPATITIS COINFECTIONS

### 2.8.0 TREATMENT GOAL OF HIV-1 INFECTION

The primary goal of HAART according to Volberding and others (2010), is to suppress HIV-1 RNA lower than the detection level (LDL) of the assay within 3 to 6 months on therapy and

improve the immune system, to lessen morbidity and mortality, to reduce vertical transmission, and restore quality of life.

### 2.8.1 THE IMPACT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON HIV-1 POSITIVE PATIENTS

In recent years (mid-1990s), highly active antiretroviral therapy (HAART) has become the backbone in the management of HIV positive individuals. It is a composition of at least three different antiretroviral agents (Ramirez-Amador *et al*, 2003; Arts *et al*, 2012). The use of agents from different classes is important in the control of resistance. It is difficult for HIV to become drug-resistant when multiple antiretroviral drugs with varied mechanisms of action are combined into a single treatment which presently stands as the hallmark of HAART.

HAART has caused a profound suppression in viral replication, increase in CD4 cell and improve their function, re-establishing the immunity of the host and improving chances of survival and decreased death rates among HIV positive patients (Hammer *et al*, 1996; Ho, 1996; Chaisson *et al*, 1997). A marked reduction in morbidity has also been credited to the introduction and the use of HAART regimens (Palella *et al*, 1998).

Also, studies elsewhere had revealed a drastic reduction in HIV/AIDS related opportunistic infections, average rate in hospitalization and the general improvement of health among HIV infected patients in the advent of HAART (Eyeson *et al*, 2002; Ramirez-Amador *et al*, 2003; Chakraborty, 2004). The declines in hospitalizations and deaths have resulted in considerable reduction in general health care costs associated with HIV infected patients.

### 2.8.2.1 FIVE MAIN CLASSES OF HAART REGIMEN

There are five distinct classes of antiretroviral drugs currently in use for the treatment of HIV infection. These agents act at different stages in the replication cycle of the virus (Clavel and Hance, 2004).

**2.8.2.1.1 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIS)** — This class of drugs inhibit the reverse transcriptase (RT) enzyme which goes to prevent HIV from copying itself. NNRTIs bind in a hydrophobic pocket close to the active site of reverse transcriptase, and restrict the conformational change needed for the catalytic activities of the RT. Examples are Efavirenz, Nevirapine and Rilpivirine (Clavel and Hance, 2004)

**2.8.2.1.2 Protease Inhibitors (PIS)** — PIs target and inhibit protease, an enzyme HIV requires to copy itself and also required for the cleavage of precursor proteins (*gag and gag–pol*) into functional proteins, which assists in the final assembly of the viral particle. The blocking of protease does not allow HIV to infect new cells. This reduces the amount of virus in the blood. Examples are Atazanavir, Fosamprenavir, Indinavir, Lopinavir/ritonavir, Nelfinavir, Ritonavir etc (Hartman and Buckheit, 2012).

**2.8.2.1.3 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIS)** — This class of inhibitors are fused into the DNA of HIV, which prevents the virus from copying itself. They terminate DNA chains and inhibit reverse transcription of the viral RNA genome into

DNA. This class serves as the backbone of HAART. Examples are Abacavir, Didanosine, Emtricitabine, Lamivudine (3TC), Stavudine (d4T), Tenofovir, and Zidovudine (AZT) (Clavel and Hance, 2004).

**2.8.2.1.4 Integrase Inhibitors (INSTIS)** — They inhibit the action of integrase. Integrase is an enzyme that HIV requires to make copies of itself and responsible for incorporating HIV DNA into the host cell DNA for transcription of the viral mRNA. Example is Raltegravir (MK-0518) (Arts and Hazuda, 2012).

**2.8.2.1.5 Entry Inhibitors-** They function by antagonizing the CD4 receptors, co-receptors and the fusion of the virus to the cell and also prevent the entry of HIV into the CD4 cells. Examples are Marviroc and Enfuvirtide (Clavel and Hance, 2004).

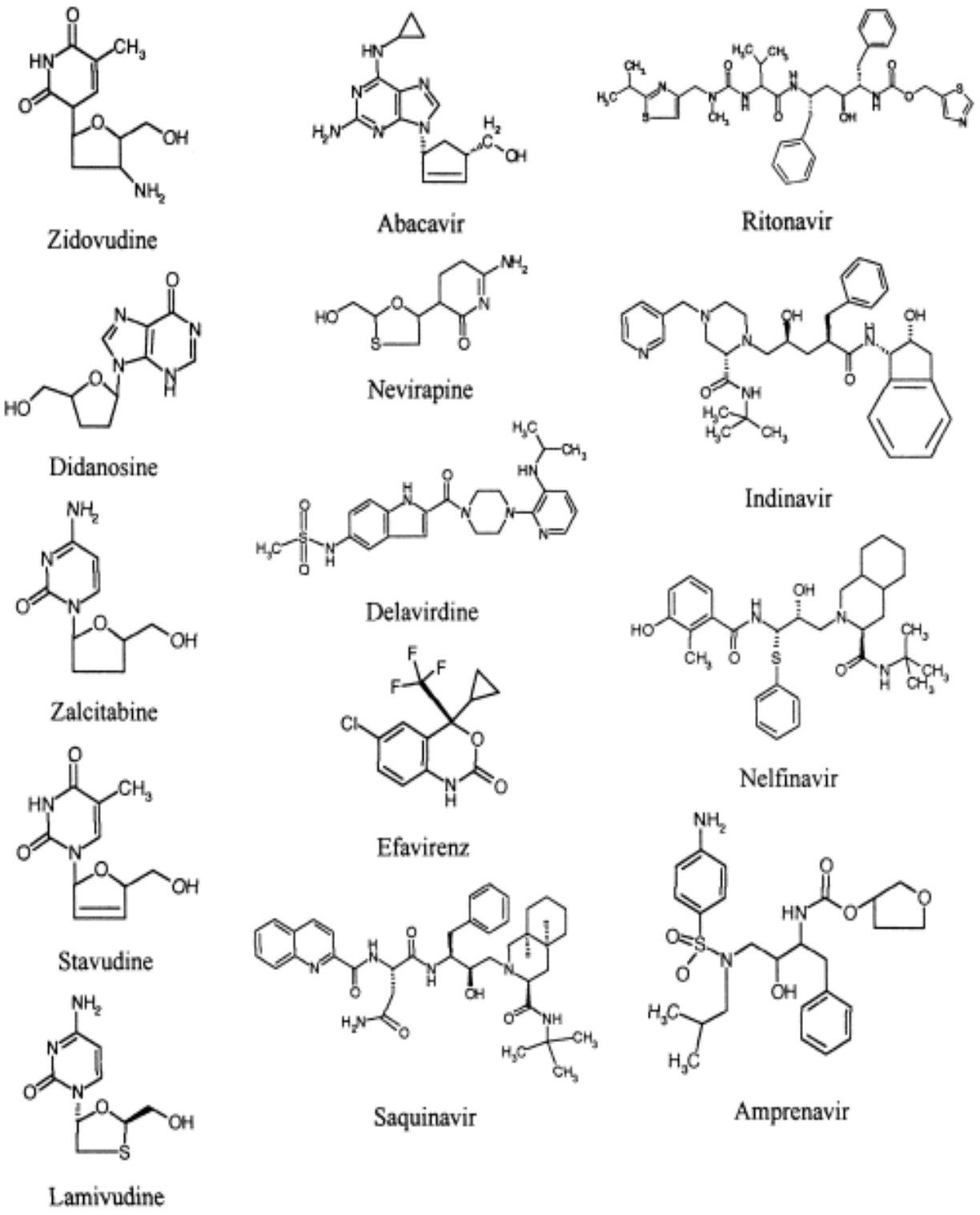


**Figure 4: Chemical structures of some antiretroviral drugs (Source: Izzedine *et al,* 2001)**

### 2.8.4 TREATMENT GOALS OF VIRAL HEPATITIS IN HIV POSITIVE PATIENTS

The ultimate goal of treatment in this group of patients is to modify anti-HIV therapy in HIV/HBV/HCV co-infected patients and yet boost immune function and reduce HIV related conditions. Unfortunately, the lack of monitoring and treatment of hepatitis has led to a wide gap being created between the real-life situation on the ground in resource-limited countries and international recommendations and guidelines (WHO, 2010). HIV patients co-infected with hepatitis are to commence HAART irrespective of their CD4 counts (Thompson *et al*, 2010).

#### 2.8.4.1 TREATMENT OF HBV AND HCV IN HIV CO-INFECTED PATIENTS

The fundamental goal of HBV treatment is to cause suppression in the replication of HBV. Even with treatment, complete resolution of the HBV indicated by seroconversion to HBsAb from HBsAg is rare. Treatment among HIV/HBV co-infected patients therefore remains unlimited (Lok *et al*, 2004; Keeffe *et al*, 2006).

Drugs such as interferon alpha (standard or pegylated), lamivudine, adefovir, entecavir. tenofovir and emtricitabine with anti-HBV activity have been recommended and approved for use among co-infected patients (Peters *et al*, 2005).

In HCV infection, the treatment is to help achieve a sustained virologic response (SVR). SVR is an undetectable level of serum HCV RNA after the end of 6 months of therapy. The recommended and approved direct acting antiviral (DAA) agents currently in use for the treatment HCV are simeprevir, sofosbuvir and ledipasvir. These agents can be used alongside

pegylated interferon alfa (PegIFN) and RBV to attain high SVR rates and very convenient to patients (Afdhal *et al*, 2014; Kowdley *et al*, 2014).

#### 2.8.4.2 TREATMENT OF TRIPLE INFECTION (HIV/HBV/HCV)

Triple infection with HBV/HCV/HIV poses a complex clinical presentation. This is so because of the interaction of HBV and HCV, and the pronounced effect of HIV on the immune system.

It is important to mention, that no standard treatment exists for co-infected patients with these triple viruses. Meanwhile, management and care of this condition must be tailored with the individual in mind and well-coordinated with an HIV specialist. It is instructive that HIV infection must be controlled prior to treatment of viral hepatitis (Crockett *et al*, 2005)

## 2.9 LABORATORY AND CLINICAL PARAMETERS FOR MONITORING HIVDISEASE PROGRESSION AND OTHERS RELEVANT TESTS

### 2.9.1 LABORATORY MARKERS FOR HIV DISEASE PROGRESSION

CD4 cell count is recommended in the monitoring of HIV disease progression for resource poor settings (WHO, 2010), including Ghana. Even though HIV viral load testing is the gold standard used to monitor patients on HAART, its high costs and technical demands makes it unavailable for use in our setting.

Absolute CD4 lymphocyte count correlates perfectly with AIDS-defining disease, and has therefore been used to set indications for initiation and monitoring of therapy.

Stable CD4 lymphocyte counts is associated with undetectable HIV viral load in blood and decline in CD4 lymphocyte cell count correlate with increases in HIV viral load (Mellors *et al*, 1997; Juliano *et al*, 1997).

### 2.9.2 CD4 COUNT IN HIV PATIENTS

HIV primarily targets and infects immune cells called CD4 T-cells. These CD4 T-cells help coordinate the human body's immune response to infection and disease. HIV binds to receptors on CD4 cells and enters the white blood cell and commences replication in the cell (Natural Standard, 2011).

The virus replicates rapidly, leading to its abundance in the bloodstream with a rapid decline in the number of CD4 T-cells with a corresponding loss of immunity. HIV infection progresses to AIDS when CD4 cell counts drop below 200 cells/mm3 of blood which exposes the individual to greater risk of developing opportunistic infections (Natural Standard, 2011).

CD4 blood tests measure the amount of CD4 T-cells in the blood. CD4 counts of infected patients are monitored regularly to determine when to initiate HAART. It is also used to assess the immunological response of patients on HAART (NACP, 2005).

An optimum increase in CD4 count of approximately 50 to 100 cells/mm3 per year is desirable (Kaufmann *et al*, 2003).

### 2.9.3 MEASUREMENT OF WEIGHT IN HIV PATIENTS

One of the commonest presentations of HIV infection is an uncontrolled weight loss. This is indicative of disease progression and occurs at all stages of infection (Nemechek *et al*, 2000). The introduction of HAART with its positive gains has been observed by Tang and friends (2003), to have caused a significant reduction in weight loss seen among HIV patients as previously. Other studies have also stated clearly the positive achievements of HAART especially in patients with severe weight loss (Zuniga-Roiz *et al*, 2002). A study conducted in Rwanda reported that patients with good CD4 response to HAART improved in their body weights as supposed to their counterparts with poor response to therapy (Lowrance *et al*, 2009). In a recent study carried out in Ghana by Ohene *et al* (2013), recorded a median weight increase by 5.9kg and 8.0kg at 6 and 12 months after initiating ART respectively over the median baseline weight of 54kg (p-value=0.001).

The body weights of patients are therefore measured on every visit as part of clinical assessments at ART centres in Ghana. This measurement is used to assess patients' response to therapy even in the absence of CD4 and viral load results.

### 2.9.4 DIAGNOSING HBV AND HCV IN HIV PATIENTS

It is recommended that all HIV positive patients be screened for the presence of HBV by using HBsAg test as the initial serological marker (Scheiblauer, 2010) and an evaluation for HBeAg should be performed for those who test positive to determine its severity (CDC, 2013).

It is also required that every HIV positive individuals before entry into care must undertake a screening test to determine his or her HCV status (CDC, 2013). And those found to be positive

be confirmed by PCR RNA test. It is however advised that there should be regular screening for both HBV and HCV seronegative HIV positive patients since they could acquire it later in life (Danta *et al*, 2011; Sulkowski *et al*, 2011; CDC, 2014).

### 2.9.5 LIVER ENZYMES AND HAEMOGLOBIN AS MARKERS IN THE ASSESSMENT OF HEPATIC DAMAGE IN HIV-1 PATIENTS CO-INFECTED WITH HBV AND HCV

HIV and hepatic disease are independently identified to cause a wide range of haematological and biochemical changes. This is partly because of the effect of these viruses on haemopoietic cells/system and the role of the liver in haemopoiesis and coagulation (Bibas *et al*, 2011). In a review to assess the situation showed that Antiretroviral Therapy (ART) administered to AIDS patients, impact negatively on the central role of the liver in many metabolic processes thereby posing a serious risk on the patient's health (Alter, 2002).

Jones and co (2012), investigated and reported complications of HIV treatment resulting in elevations in liver enzymes following the initiation of antiretroviral therapy. Even though, recently approved antiretrovirals present a much lower risk of developing hepatotoxicity, there is a documented (Vispo *et al*, 2010) evidence of frequent flared-ups in liver transaminases among HIV patients co-infected with viral hepatitis on treatment.

Also, in Nigeria, Obi and others (2013), reported a significantly higher liver enzymes (AST, ALT, ALP) level in HIV patients co-infected with hepatotropic viruses compared with mono-infected patients. Other studies also observed marked increases in liver enzymes (ALT, AST, and Alkaline phosphates) in HIV/AIDS patients. Liver enzymes are therefore very useful

biomarkers of liver injury in individuals with some degree of intact liver function. Majority of the liver diseases cause only mild symptoms initially and must be detected early (Mgogwe *et al*, 2012).

In a review conducted by Opie to address haematological complications in HIV infection, confirmed anaemia (low Hb) to be common among HIV infected patients and worsened when co-infected with viral hepatitis (Reviewed by Opie, 2012). Again, Obi and colleagues (2013), also noticed a significantly lower Hemoglobin concentration in HIV/HCV co-infected patients compared with the HCV mono-infected control subjects which was consistent with a similar work done by Obienu and Nwokediuko (2011), all in Nigeria. These authors speculated that the resultant effect of HIV/HCV co-infection could be due to an additive or synergistic effect of the two infections. Although there are several possible causes of anemia in HIV infections, it is commonly attributed to bone marrow failure, peripheral destruction and opportunistic infections and HAART therapy (Ibeh *et al*, 2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.0 STUDY AREA

The study involved blood samples taken in the Anti-Retroviral Therapy (ART) centres of Krachi West District Hospital (KWDH), Hohoe Municipal Hospital (HMH) and the Volta Regional Hospital (VRH), all in the Volta Region of Ghana from January, 2014 to December, 2015.

The ART centres of KWDH and HMH provide ART services to clients in Krachi West District and Hohoe Municipality respectively. VRH, located in Ho, the regional capital renders ART services to patients in Ho and also serves as the major treatment centre in the Volta Region with an estimated population of over 2.2 million people.

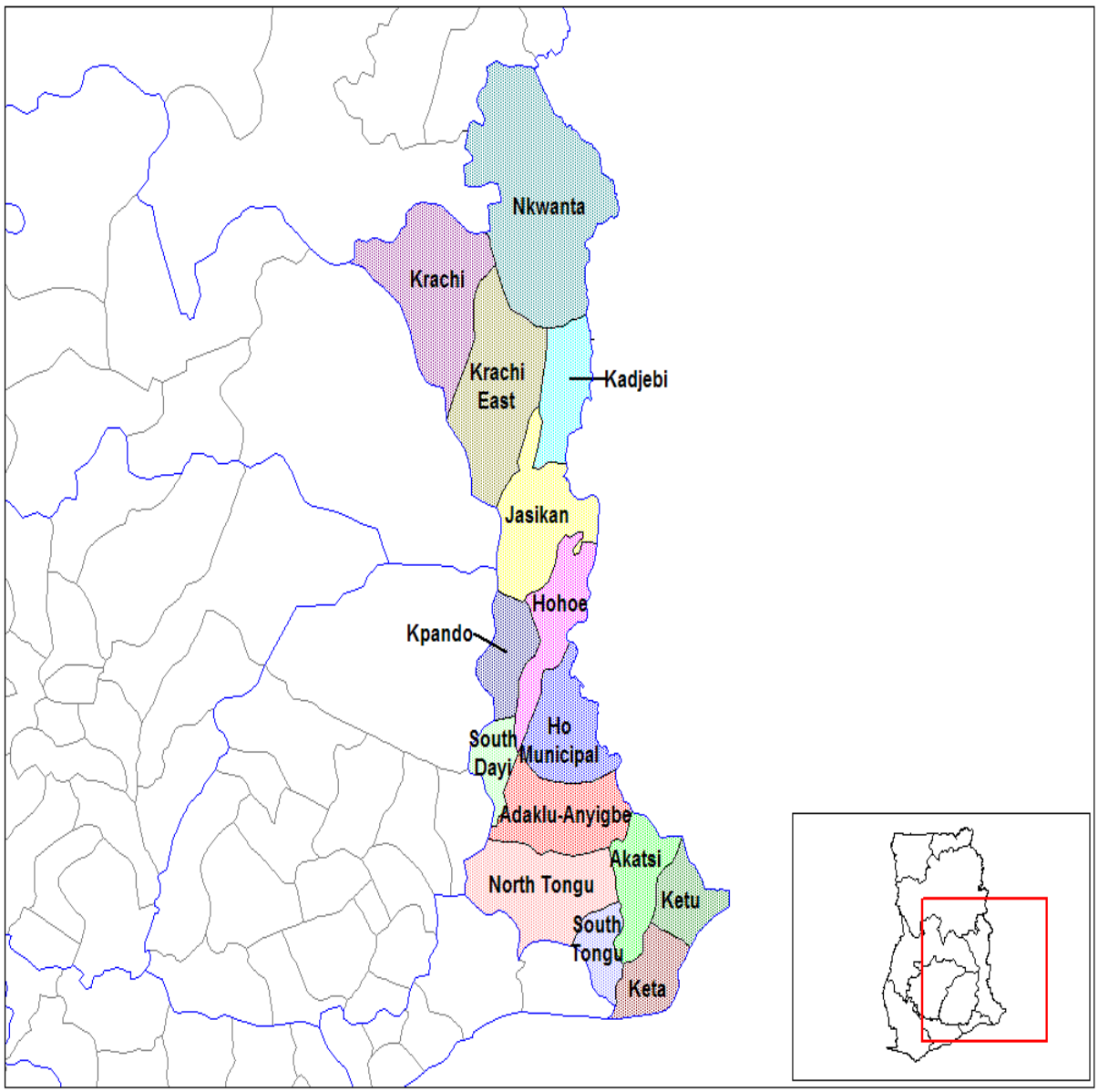


**Figure 5: The map of Ghana showing Volta Region (highlighted). Source: http://maps.google.com**

## 3.1 PATIENTS AND SAMPLES

Blood samples for this study were collected from patients attending ART centres for purposes of clinical diagnostics (referred by clinicians) and voluntary testing (walk-in). The samples were first screened for HIV-1 and those found to be reactive were later confirmed using the WHO testing protocols for HIV testing and also in line with the algorithm of the National AIDS Control Programme (NACP, 2014). The algorithm has 'First Response' as the first line HIV testing kit and 'Oraquick' as the confirmatory test kit. Patients who tested positive for HIV-1 and were eligible for treatment according to WHO/NACP criteria were selected for the study. For each eligible patient, blood sample, biographic data and other relevant information necessary for the assessment of possible risk of co-infection with HBV or HCV were obtained.

### 3.1.1 INCLUSION CRITERIA

1. All newly diagnosed HIV-1 adults (18years and above) eligible for treatment.
2. Informed written consent.

### 3.1.2 EXCLUSION CRITERIA

1. Refusal of consent.
2. Patients who tested positive to HIV- 2.
3. Patients who were already on HAART.
4. Patients with chronic alcoholism.
5. Patients who were found to be pregnant.

## 3.2 STUDY DESIGN AND SAMPLING TECHNIQUE

This work was a prospective cohort study. The study was conducted between January, 2014 and December, 2015. Samples of patients were collected on three consecutive times over a period of one year. A convenient sample size of at least 200 eligible patients (determined without employing any scientific method) who visited the three ART centres, were selected during the early periods of the study to allow time for follow-ups.

## 3.3 STUDY POPULATION

All HIV-1 positive adults who were diagnosed within the study period and satisfied the inclusion criteria were enrolled in the study.

## 3.4 DIAGNOSIS OF HIV

HIV diagnosis was carried out using HIV First Response Test Card 1–2.0 (manufactured by Premier Medical Corporation Ltd, Mumbai, India). All HIV-1 reactive blood samples were confirmed with Oraquick Rapid HIV – 1/2 (manufactured by OraSure Technologies Inc., Bethlehem, USA). These rapid test kits are immuno-chromatographic for qualitative detection of antibodies specific to HIV 1 or HIV 2 in human serum/plasma or whole blood. The principles for the tests are in appendices 1&2.

**PROCEDURE -** HIV First Response Test Card 1–2.0

The test is performed according to manufacturer's instructions.

***SERUM/PLASMA***

1. Place refrigerated or frozen sample on the bench and allow it to thaw completely and mix well prior to testing.
2. Remove device from packaging and label with client ID/ Code.
3. Collect specimen using disposable pipette included in the kit
4. Add one drop of serum/plasma to the sample port in the device.
5. Add one drop (approximately 30 µL) of diluents provided to sample in the sample port.
6. Wait for 5 – 15 minutes before reading results. (Do not read results after 15 minutes).

***WHOLE BLOOD***

1. Select a finger and clean selected area, take a good prick away from tip of finger with lancet provided and wipe off first drop of blood
2. Gently squeeze puncture area to produce a big drop of blood
3. Collect blood using disposable pipette included in the kit
4. Add one drop of whole blood to the sample port in the device
5. Add two drops (approx.. 60 µL) of diluents provided to the blood sample in the sample port
6. Wait 5 – 15 minutes before reading results. ( Do not read results after 15 minutes )

**INTERPRETATION OF RESULTS:**

**Reactive:** Appearance of 2 (two) lines in control and test areas in result window

**Reactive HIV-1:** Red line in control and point marked (1)

**Reactive HIV-2**: Red line in control and point marked (2)

**Non – Reactive:** Appearance of (1) one line in the control area and no line in the test area.

Invalid: No line appears in the control area. (Repeat test with new device even if line appears in the test areas)

## PROCEDURE - Oraquick Rapid HIV – 1/2

**Reconstituted Sample**

1. Set reusable stand on a flat, level surface. Partially remove device from package and label device and the developer vial with client ID or Code.
2. Carefully uncap the developer vial and place vial into the stand.
3. Collect approximately 5µl of specimen using a new disposal loop or a dropper.
4. Transfer the collected specimen to vial.
5. Stir the specimen in the vial with a loop or dropper.
6. Insert the device pad completely into the vial with the result window facing forward.
7. Wait between 20–40 minutes before reading results. (Do not read results after 40 minutes).
8. Read and record the results.

**INTERPRETATION OF RESULTS**

**Reactive:** Appearance of lines of any intensity in both the control and test areas.

**Non – reactive:** Appearance of one line in the control area only

**Invalid:** No line appears in the control area.

### 3.5 COLLECTION OF WHOLE BLOOD SAMPLE

Sterile 10ml Becton Dickinson (BD) syringes were used to collect 10ml of blood sample from each of the 200 HIV-1 positive patients eligible for treatment. Five ml of the blood samples were immediately dispensed into sterile BD vacutainer tubes containing ethylene-diamine-tetra-acetic acid (EDTA) to prevent clotting. The blood in the EDTA tubes was allowed to mix thoroughly on a blood mixer which was used for CD4 count and haemoglobin estimation immediately. The remaining 5ml blood samples were dispensed into BD clinical chemistry vacutainer tubes with serum separator gel. The samples collected in the chemistry tubes were later centrifuged at 3000rpm for 2 minutes. The serum was then separated into plain tubes using Pasteur's pipette for the determination of the presence of HBsAg and anti-HCV and the estimation of liver enzymes (ALT, AST and ALP). All sample tubes were coded and serum stored at -20 degrees celcius. The stepwise sampling procedure is in appendix 3.

### 3.6 LABORATORY EXAMINATION

Before enrolment into the study, participants were screened and confirmed for HIV-1 by the investigator.

#### 3.6.1 HBV/HCV SCREENING

The coded samples were tested for the presence of HBsAg for HBV (test kit manufactured by bio Merieux, France) and anti-HCV antibodies for HCV (Flavicheck-HCV, test kit manufactured by Qualpro Diagnostics, India). Determination for the presence of HBsAg and antibodies to HCV were performed using a third and fourth generation EIA (an enzyme

immunoassay test) respectively, as they have a sensitivity of greater than 99% in high-risk populations. The sera of patients were screened for HBsAg and anti-HCV antibody under the supervision of the Senior/Principal Biomedical Laboratory Scientists in charge of the respective ART laboratories. The manufacturer's instructions were strictly adhered. The Flavicheck- HCV was designed for the total detection of antibodies specific to HCV in serum or plasma. Whereas HBsAg test kit was targeted at detecting the surface antigens to HBV. The procedure and interpretation of HBV are the same with that of HCV.

Stepwise standard operational procedures (SOPs) were strictly adhered in answer to quality control (QC) issues. Known HBsAg and anti-HCV antibody positive and negative control samples were routinely used to control the test kits.

**Interpretation of test results**

Two red visible bars in the control and patients windows of the test strip signify a positive test result, while the appearance of only one bar in the control window indicated negative result.

### 3.6.2 CD4 COUNT

The CD4 count of all the HIV-1 positive participants was measured on the same day of sampling using the CD4 automated machine (BD FACSCount). The machine employs the principle of flow cytometry (appendix 4). The quality of CD4 counts were monitored routinely by running control beads or materials before the measurement of study samples.

**Figure 6: BD FACSCount instrument**

### 3.6.3 MEASUREMENT OF LIVER ENZYMES

A fully Automated Chemistry Analyzer (Vitalab Selectra Junior, Germany) was used to measure the following liver enzyme levels: AST, ALT and ALP in the coded serum samples after daily calibration of analyzer. The equipment operates on the principle based on the fact that substances of interest are selectively absorb or emit energy (light) at different specified wavelengths. The determination of AST and ALT, are based on the AST catalyzed transfer of amino group of L-aspartate to alpha-ketoglutamate to form L-glutamate and ALT catalyzed transfer of amino group of L-alanine to alpha-ketoglutarate to form L-glutamate (Schuman *et*

*al*, 2002) whiles, Alkaline Phosphatase was based on its catalyzed conversion of p-nitrophenylphosphate to p-nitrophenol and inorganic phosphate.

### 3.6.4 MEASUREMENT OF HAEMOGLOBIN

Haemoglobin was estimated using the Sysmex KX21N analyzer (Sysmex Corporation, Kobe, Japan). Even though the fully automated haematology analyzer has the capacity to measure 18 parameters, this study only extracted the haemoglobin results. Haemoglobin concentration was estimated using a more reliable and accurate non-cyanide haemoglobin method. For quality control purposes, three controls (Low, Normal and High) were run daily. Blood samples of participants were processed only when the QC material employed had passed.

### 3.6.5 MEASUREMENT OF WEIGHT

The body weight of each patient was weighed to the nearest 1.0 kg in light clothing. The accuracy of the bathroom scale was assessed using a standardized weight and a subsequent visual inspection for zero-balancing at the beginning of each day's measurement.

## 3.7 ANTI-RETROVIRAL TREATMENT INITIATION AND MONITORING

WHO recommendations on initiation of ART were followed: HIV-1 positive patients with CD4 cell count <350cells/μL or its equivalent WHO staging III & IV were eligible and therefore put on treatment (HAART). First line standard combination regimen comprising zidovudine (300mg), lamivudine (150mg) and nevirapine (200mg) was served. In case of

contraindications to zidovudine or nevirapine, tenofovir (300mg) or efavirenz (600mg) was given. All subjects with haemoglobin counts equal or higher than 8g/dl were started on zidovudine, lamivudine and nevirapine. Subjects who were anaemic (haemoglobin counts less than 8g/dl) at the time of recruitment (4 subjects) or those who developed severe anaemia (3 subjects) during the course of the above-said regimen were given a combination regimen of tenofovir, lamivudine and nevirapine. There are other times where the drug combination is based on availability. Hepatitis co-infection was not considered in the choice of drugs simply because the hepatitis status of the patients was unknown to the healthcare providers during the study period.

Prior to the initiation of treatment, all patients received comprehensive adherence counseling and issues related to other medical conditions were equally addressed. Adherence assessment was based on pill counts and interviewing of patients (self-report) at every visit.

Various laboratory examinations including liver enzymes, haematology (Hb) and CD4 cell count were done prior to start of treatment (HAART). After HAART initiation, Hb, liver enzymes (ALT, AST, ALP), and CD4 cell count were repeated twice at six month intervals.

The body weights of the participants were also taken at the start of treatment, and thereafter, six monthly to the twelve month.

### 3.7.1 RESPONSE OF HBV/HCV CO-INFECTED PATIENTS FOLLOWING HAART

The primary outcome of interest in this study was the immunological and clinical response to HAART. Immunologic response was measured by CD4 cell count and the immunological development or progression was determined by average changes in CD4 counts from baseline through to 12 months. Clinical response to HAART was also assessed based on the average changes in body weight from start of treatment to 12 months. Hepatotoxicity was defined as patients with transaminases; ALT, AST and ALP rise above the upper limits of the normal values, according to the AIDS Clinical Trials Group scale of liver toxicity (AIDS Clinical Trials Group, 1996).

Results or data from eligible patients were used in the analysis of the study. The study participants were grouped into HIV alone, HIV/HBV only and HIV/HCV only. Baseline or initiation point was defined as the day the study participant commenced treatment. On the start day of treatment, patients underwent a complete clinical assessment (including body weight measurement) and baseline laboratory tests (haemoglobin, liver enzymes, and CD4 cell count). Two similar follow-ups were done later and results included in the analysis.

## 3.8 COVARIATES OF INTEREST

Demographic information and variables related to HIV disease progression were taken as possible confounders through the administration of a structured questionnaire to each participant. Covariates tested for inclusion were age, sex, ethnic group, religion, economic

status, education and HIV exposure categories such as men who have sex with fellow men (MSM), injection drug use (IDU), heterosexual sex, blood transfusion and prostitution among others.

### 3.9 DATA ANALYSIS

In the initial analysis, the hepatitis prevalence rate among HIV positive patients was calculated from the total numbers that were serologically tested for both HBV and HCV. Normality of all continuous variables was tested and non-conforming variables were normalized by log transformation before analysis and results converted by anti-log where appropriate. Continuous variables were expressed as their mean ± SD, whereas categorical variables were expressed as figure and proportion. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant. The comparism of mean of hepatitis positive and negative individuals was done using unpaired student t-test. Paired analysis of variance and Benferroni post-hoc test were used to compare mean variables for baseline, month six and month twelve. Comparisms of trends using categorical outcomes were done using chi-square test for trends.

### 3.10 ETHICAL APPROVAL

The Committee on Research, Publication and Ethics of the KNUST Medical School gave approval for the study to be undertaken (Ethical Clearance Ref No: CHRPE/AP/012/14) (Appendix 5). Permission was also sought and obtained from the management of the selected

hospitals. Written informed consent form was obtained from each participant. Participants were fully informed about the purpose, procedures, risks and benefits of participating in this study. Those who agreed to participate signed or thumb printed the informed consent form (Appendix 6). Each participant was assured that their responses would be kept confidential and data collected will be kept for the purpose of this study only.

# CHAPTER FOUR

# RESULTS

## 4.1 PATIENT CHARACTERISTICS

Between January 2014 and December 2015, 1978 adults representing clinical diagnostic and voluntary counseling and testing clients who presented at the various ART centres were tested for HIV. Of these, 315 were confirmed HIV positive (prevalence of 15.9% among this group). Two hundred of the HIV-1 positive patients who met the study criteria were recruited and included in the study population and screened for the presence HBV and HCV. Out of this number, 110 participants had results for all the parameters at baseline and the two follow-ups which were used in the analysis.

Among the 200 HIV-1 seropositives who participated in the current study, majority (70%) were females. The average age of a participant was 39 years, ranging from a minimum of 18 years to 68 years. More than 52% were married and Christianity was found to be the dominant religious inclination (88.5%). Thirty four percent of the respondents had attained at least secondary level education at the point of the study. Majority of the study participants were gainfully employed (73%), predominately in the informal sector (55%) and fell within the low income bracket (66.5%). More than half (57%) resides in the urban areas. No intravenous drug user (IVDU), prostitution and men who had sex with men (MSM), was recorded among the study population.

**Table 1: Socio-Demographic Characteristic of participants presenting with HIV in selected ART centres in the Volta Region of Ghana**

| Parameter | Frequency | Percentage (%) |
|---|---|---|
| Total Respondent | 200 | 100.0 |
| **Age** | | |
| **18-20** | 4 | 2.00 |
| **21-30** | 38 | 19.00 |
| **31-40** | 69 | 34.50 |
| **41-50** | 69 | 34.50 |
| **>50** | 20 | 10.00 |
| **Gender** | | |
| Male | 60 | 30.00 |
| Female | 140 | 70.00 |
| **Marital Status** | | |
| Single | 95 | 47.50 |
| Married | 105 | 52.50 |
| **Religious Status** | | |
| Christian | 177 | 88.50 |
| Islam | 21 | 10.50 |
| Traditionalist | 2 | 1.00 |
| **Educational Background** | | |
| No Education | 47 | 23.50 |
| Basic | 84 | 42.00 |
| Secondary | 45 | 22.50 |
| Tertiary | 24 | 12.00 |
| **Employment Status** | | |
| Unemployed | 54 | 27.00 |
| Informal Sector | 110 | 55.00 |
| Formal Sector | 36 | 18.00 |
| **Economic Status** | | |
| Low Income | 133 | 66.50 |
| Middle Income | 64 | 32.00 |
| High Income | 3 | 1.50 |
| **Area of Residence** | | |
| Urban | 114 | 57.00 |
| Rural | 86 | 43.00 |

Data is presented as frequency and proportions

## 4.2 SEROPREVALENCE OF HBV AND HCV

As depicted in Table 2 below, hepatitis viral co-infection was 8.5% among the study respondents. Seven percent (7%) of participants tested positive for hepatitis B virus and 3 participants representing 1.5% of the study population tested positive to hepatitis C virus. However, none of the subjects was co-infected with both hepatitis B and C. Hepatitis co-infection was descriptively higher among female (9.29%) and married (9.52%) participants. Percentage hepatitis viral co-infection was highest in respondents without any formal education and those who were not gainfully employed at the time of the study. Urban duelers (9.65%), those who had ever experienced blood transfusion (15.79%) and those who have ever shared sharps (8.94%) recorded higher percentage co-infection.

**Table 2: Hepatitis viral co-infection profile of HIV seropositives attending ART centres in the Volta Region of Ghana**

| Parameter | Frequency | Percentage |
|---|---|---|
| Prevalence Hepatitis Virus | 17 | 8.50 |
| HBV | 14 | 7.00 |
| HCV | 3 | 1.50 |
| **Age Distribution** | | |
| 18-30 | 6 | 35.28 |
| 31-40 | 5 | 29.40 |
| 42-68 | 6 | 35.28 |
| **Gender** | | |
| Male | 4 | 23.56 |
| Female | 13 | 76.44 |
| **Marital Status** | | |
| Single | 7 | 41.20 |
| Married | 10 | 58.80 |
| **Religious Status** | | |
| Christian | 15 | 88.24 |
| Islam | 2 | 11.76 |
| Traditionalist | 0 | 0.00 |
| **Educational Background** | | |
| No Education | 5 | 29.40 |
| Basic | 8 | 47.08 |
| Secondary | 2 | 11.76 |
| Tertiary | 2 | 11.76 |
| **Occupational Status** | | |
| Unemployed | 6 | 35.28 |
| Formal Sector | 2 | 11.76 |
| Informal Sector | 9 | 52.96 |
| **Area of Residence** | | |
| Urban | 11 | 64.72 |
| Rural | 6 | 35.28 |
| **Transfusion Status** | | |
| Ever been transfused | 3 | 17.64 |
| Never been transfused | 14 | 82.36 |
| **Sharing of sharps with other** | | |
| Ever share sharps | 11 | 64.72 |
| Never share sharps | 6 | 35.28 |

Data is presented as frequency and proportions

### 4.3 DISEASE PROGRESSION AND TREATMENT OUTCOME

Among the general study population, significant appreciable levels of CD4, weight and haemoglobin were observed from the baseline estimates through the sixth month estimates to the twelve months of HAART therapy. From Table 3, significant increase in ALT levels from the baseline was observed after six month of HAART treatment and remained statistically stable afterward. No significant change of AST levels was observed during the course of HAART therapy. ALP levels rose significantly during the first sixth month of therapy and fell to the level of HAART naïve during the next sixth months of therapy. Table 3.

**Table 3: Treatment outcome among HIV seropositive receiving HAART therapy at ART centres in the Volta Region of Ghana**

| Parameter | Baseline | Month-6 | Month-12 | *P* | *p1* | *p2* | *p3* |
|---|---|---|---|---|---|---|---|
| CD4 | 133.78±3.22 | 231.24±1.9 | 303.73±1.77 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Weight | 56.60±9.02 | 59.12±8.51 | 60.28±8.2 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Haemoglobin | 10.95±1.96 | 11.47±1.71 | 12.04±1.41 | < 0.0001 | 0.0014 | < 0.0001 | 0.0001 |
| ALT | 24.09±10.24 | 27.04±13.08 | 27.67±12.39 | 0.0041 | 0.0424 | 0.0077 | 0.7776 |
| AST | 26.18±10.67 | 28.65±11.73 | 27.89±9.87 | 0.0735 | 0.1184 | 0.2877 | 0.5846 |
| ALP | 76.79±29.11 | 83.95±30.19 | 78.97±21.46 | 0.0153 | 0.0246 | 0.6074 | 0.1146 |

Data is presented as mean±standard deviation. P-value is significant at 0.05. p compare all mean (ANOVA), p1 compares baseline with month six, p2 compares baseline with month 12 and p3 compares month six with month twelve. ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP-Alkaline phosphatase.

Among the hepatitis virus naïve participants, significant appreciable levels of CD4, weight and haemoglobin were observed from the baseline estimates through the sixth month estimates to the twelve months of HAART therapy. However as observed from Table 4, the hepatitis virus co-infected group presented no significant change in the aforementioned variables. In case of CD4 levels, the two groups presented with comparable levels at baseline, but the hepatitis virus naïve group recorded a significantly higher CD4 levels after six months and at twelve month of therapy compared to the hepatitis co-infected group. Weight gain among the mono-infected HIV group saw a significant increase as compared to the hepatitis co-infected group. Haemoglobin concentration was comparable between the two groups of HIV patients at baseline through to the first six month of therapy, however the hepatitis virus naïve group presented a significantly higher haemoglobin levels at the end of the twelve months of treatment. No significant change in the levels of enzymes was observed among the two groups during the course of the study.

**Table 4: Treatment outcome among HIV seropositive receiving HAART therapy at the ART centres in the Volta Region stratified by hepatitis virus co-infection (HBV/HCV)**

| | | Parameter | Baseline | Month-6 | Month-12 | *p-value* |
|---|---|---|---|---|---|---|
| **CD4 count** | **(cells/ml)** | **Hepatitis –Negative** | 130.59±3.35 | 250.21±1.8 | 333.73±1.664 | *<0.0001* |
| | | **Hepatitis-Positive** | 152.69±2.59 | 150.24±2.12 | 181.43±1.854 | *0.7417* |
| | | ***p-value*** | *0.6148* | *0.0022* | *<0.0001* | |
| **Weight** | **(kg)** | **Hepatitis –Negative** | 56.18±8.54 | 59.02±8.1 | 60.46±8.086 | *0.0018* |
| | | **Hepatitis-Positive** | 58.88±11.36 | 59.94±11.12 | 59.29±8.964 | *0.9573* |
| | | ***p-value*** | *0.2585* | *0.6864* | *0.5912* | |
| **Haemoglobin** | **(g/dl)** | **Hepatitis –Negative** | 11.00±1.97 | 11.57±1.75 | 12.25±1.287 | *<0.0001* |
| | | **Hepatitis-Positive** | 10.64±1.9 | 10.93±1.38 | 10.93±1.572 | *0.8388* |
| | | ***p-value*** | *0.4832* | *0.1581* | *0.0003* | |
| **ALT conc** | **(U/L)** | **Hepatitis –Negative** | 23.18±10.07 | 25.81±12.93 | 27.04±12.445 | *0.0792* |
| | | **Hepatitis-Positive** | 29.06±9.97 | 33.76±12.14 | 31.12±11.837 | *0.4855* |
| | | ***p-value*** | *0.0289* | *0.0204* | *0.2140* | |
| **AST conc** | **(U/L)** | **Hepatitis –Negative** | 26.25±10.95 | 28.39±11.91 | 27.68±10.089 | *0.4031* |
| | | **Hepatitis-Positive** | 25.82±9.28 | 30.12±10.87 | 29.06±8.785 | *0.4106* |
| | | ***p-value*** | *0.8811* | *0.5783* | *0.5981* | |
| **ALP conc** | **(U/L)** | **Hepatitis –Negative** | 78.19±29.65 | 85.66±30.72 | 80.19±21.165 | *0.1618* |
| | | **Hepatitis-Positive** | 69.12±25.37 | 74.59±25.94 | 72.29±22.494 | *0.8104* |
| | | ***p-value*** | *0.2389* | *0.1656* | *0.1639* | |

Data is presented as mean ± standard deviation. P-value is significant at 0.05. ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, ALP- Alkaline phosphate.

Among the hepatitis B virus naïve participants, significant appreciable levels of CD4, weight and haemoglobin were observed from the baseline estimates through the sixth month estimates to the twelve months of HAART therapy in contrast hepatitis B co-infected patients showed no significant increase of CD4, weight and haemoglobin levels throughout the twelve months of HAART therapy.

No significant changes in the concentrations of all three liver enzymes assayed in this study were recorded for both hepatitis B virus seropositive and seronegative participants. Table 5.

**Table 5: Treatment outcome among HIV seropositvie receiving HAART therapy atthe ART centres in the Volta Region stratified by hepatitis B viral co-infection**

| Parameter | | | Baseline | Month-6 | Month-12 | *p-value* |
|---|---|---|---|---|---|---|
| **CD4 count** | **(cells/ml)** | **HBV-Negative** | 132.07±3.31 | 248.08±1.81 | 329.99±1.67 | *<0.0001* |
| | | **HBV-Positive** | 146.22±2.73 | 142.89±2.17 | 172.07±1.88 | *0.8079* |
| | | ***p-value*** | *0.7625* | *0.0023* | *<0.0001* | |
| **Weight** | **(kg)** | **HBV-Negative** | 56.54±8.71 | 59.44±8.31 | 60.66±8.06 | *0.0025* |
| | | **HBV-Positive** | 57.00±11.31 | 57.29±10.39 | 57.71±8.94 | *0.9830* |
| | | ***p-value*** | *0.8600* | *0.3831* | *0.2110* | |
| **Haemoglobin** | **(g/dl)** | **HBV-Negative** | 10.95±1.97 | 11.52±1.75 | 12.17±1.34 | *<0.0001* |
| | | **HBV-Positive** | 10.93±1.98 | 11.13±1.43 | 11.19±1.61 | *0.9143* |
| | | ***p-value*** | *0.9727* | *0.4278* | *0.0142* | |
| **ALT conc** | **(U/L)** | **HBV-Negative** | 23.40±10.01 | 26.42±13.25 | 27.52±12.61 | *0.0503* |
| | | **HBV-Positive** | 28.86±10.9 | 31.29±11.42 | 28.71±11.08 | *0.7911* |
| | | ***p-value*** | *0.0619* | *0.1947* | *0.7380* | |
| **AST conc** | **(U/L)** | **HBV-Negative** | 26.31±10.81 | 28.90±12.1 | 28.07±10.21 | *0.2573* |
| | | **HBV-Positive** | 25.29±9.93 | 27.00±8.91 | 26.64±7.29 | *0.8623* |
| | | ***p-value*** | *0.7382* | *0.5745* | *0.6149* | |
| **ALP conc** | **(U/L)** | **HBV-Negative** | 78.74±29.39 | 86.34±30.68 | 80.93±21.27 | *0.1434* |
| | | **HBV-Positive** | 63.43±23.95 | 67.50±20.74 | 65.57±18.27 | *0.8782* |
| | | ***p-value*** | *0.0657* | *0.0285* | *0.0117* | |

Data is presented as mean±standard deviation. P-value is significant at 0.05. ALT-Alanine aminotransaminase, AST-Aspartate aminotransaminase, ALP-Alkaline phosphatase

Patients with no co-infection with HCV exhibited significant additive levels of CD4, haemoglobin and weight gain during the course of HAART therapy whilst those co-infected with HCV showed no significant change in CD4, Weight and Haemoglobin levels at the end of twelve months of HAART therapy though no significant difference was observed at the initiation of treatment.

**Table 6: Treatment outcome among HIV seropositvie receiving HAART therapy at the ART centres in the Volta Region stratified by hepatitis C viral co-infection**

| Parameter | | Baseline | Month-6 | Month-12 | *p-value* |
|---|---|---|---|---|---|
| **CD4 count (cells/ml)** | **HCV-Negative** | 132.53±3.26 | 232.54±1.9 | 305.98±1.77 | *<0.0001* |
| | **HCV-Positive** | 186.90±2.2 | 189.93±2.05 | 232.27±1.78 | *0.9154* |
| | ***p-value*** | *0.6182* | *0.5922* | *0.4102* | |
| **Weight (kg)** | **HCV-Negative** | 56.29±8.89 | 58.79±8.4 | 60.10±8.21 | *0.0042* |
| | **HCV-Positive** | 67.67±7.77 | 72.33±2.89 | 66.67±4.93 | *0.4604* |
| | ***p-value*** | *0.0306* | *0.0065* | *0.1723* | |
| **Haemoglobin (g/dl)** | **HCV-Negative** | 10.99±1.97 | 11.51±1.71 | 12.11±1.37 | *<0.0001* |
| | **HCV-Positive** | 9.30±0.53 | 10.00±0.7 | 9.73±0.64 | *0.4383* |
| | ***p-value*** | *0.141* | *0.1321* | *0.0036* | |
| **ALT conc (U/L)** | **HCV-Negative** | 23.93±10.31 | 26.52±12.83 | 27.26±12.24 | *0.0977* |
| | **HCV-Positive** | 30.00±4.58 | 45.33±9.29 | 42.33±9.71 | *0.1284* |
| | ***p-value*** | *0.313* | *0.0134* | *0.0371* | |
| **AST conc (U/L)** | **HCV-Negative** | 26.12±10.78 | 28.21±11.54 | 27.54±9.75 | *0.3488* |
| | **HCV-Positive** | 28.33±6.03 | 44.67±6.66 | 40.33±6.35 | *0.0468* |
| | ***p-value*** | *0.7249* | *0.0158* | *0.0262* | |
| **ALP conc (U/L)** | **HCV-Negative** | 76.26±29.3 | 83.28±30.16 | 78.28±21.31 | *0.1534* |
| | **HCV-Positive** | 95.67±11.68 | 107.67±24.38 | 103.67±9.29 | *0.6802* |
| | ***p-value*** | *0.2566* | *0.1687* | *0.0428* | |

Data is presented as mean ± standard deviation. P-value is significant at 0.05. ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP-Alkaline phosphatase

## 4.4 IMPACT OF HEPATITIS CO-INFECTION ON OUTCOME OF HAART

As seen in Figure 7A, at the initiation of therapy, 47.27% and 52.73% of respondents clustered at CD4 levels of less than 200 and from 200 to 500 respectively. Cluster distribution among hepatitis virus naïve and hepatitis virus co-infected were comparable at baseline and after six months of HAART therapy (Figure 7A &B). Significant shift in cluster distribution across the three different CD4 categorizations was observed at twelve months of HAART experience between patients with hepatitis co-infection and those with no hepatitis infection (p-0.0006). Among the co-infected group 52.94% clustered below 200 and the rest at 200 to 500 with none crossing the 500 mark. For the hepatitis negative group 26.88% crossed the 500 mark, with 58.06 clustering at 200 to 500 and 20.91% clustering below the 200 mark, Figure 7C.

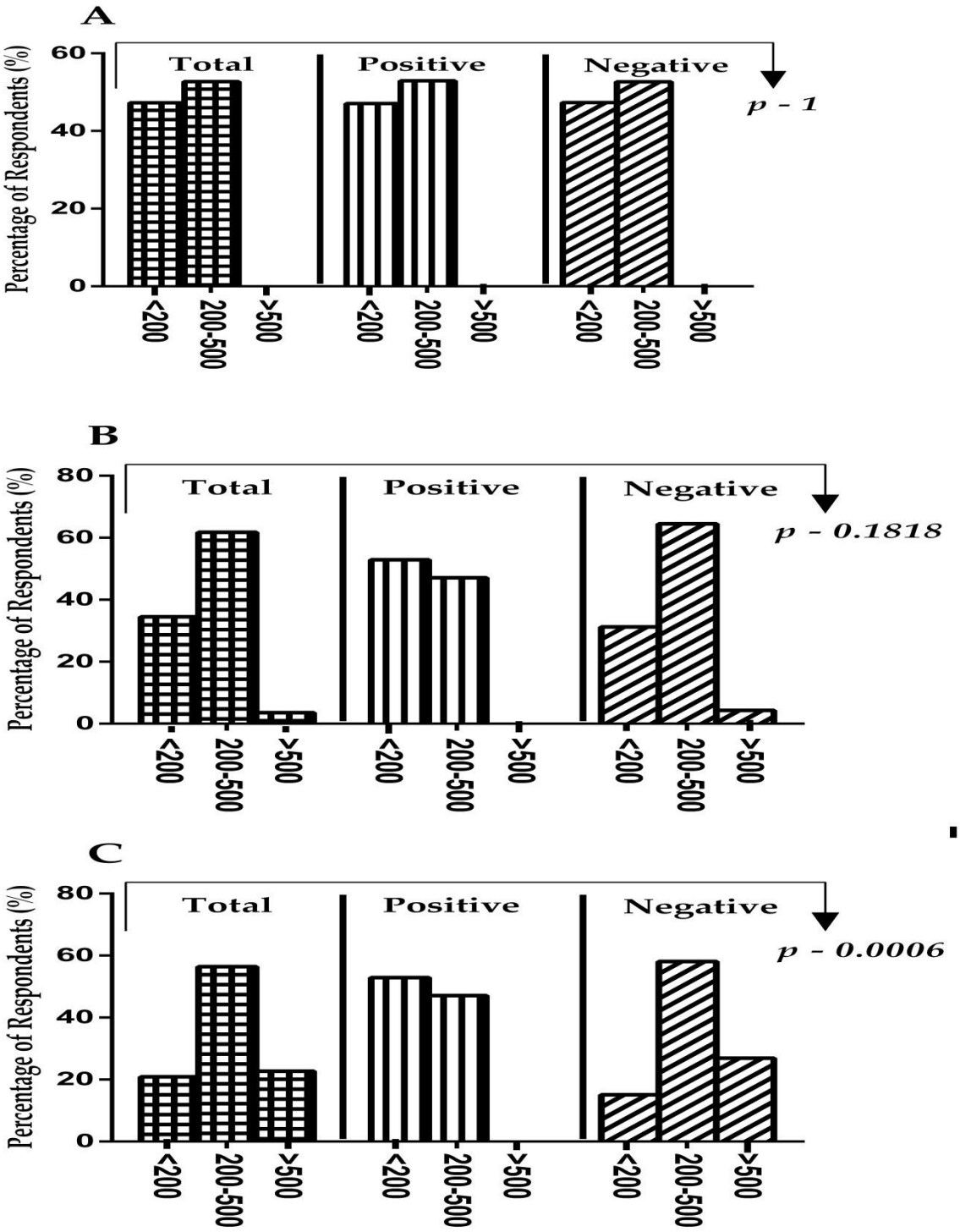


**Figure 7: Respondent CD4 clusters stratified by hepatitis viral co-infection. A-Baseline, B- Month six and C-Month twelve**

Classification by treatment outcome into, no change in CD4, decrease in CD4, or increase in CD4 levels from initiation to the end of 12 months revealed that 10.91% of the total study population experienced reduction in their CD4 levels with the rest experiencing improvement in immunity (89.09%). Compared to those who tested negative to hepatitis, only 5.38% witnessed a reduction in CD4 levels whereas a significant proportion of hepatitis positive individuals (41.18%) deteriorated in immunity (p-0.0003), Figure 8A.

As seen in Figure 8B, 4.55% of the total population experienced no change in weight, 10.90% lost weight and 84.55% gained weight at the end of 12 months of treatment. Difference in weight outcome cluster distribution was observed among the hepatitis positive and negative group with higher weight gain clustering among the hepatitis naïve (84.55%) compared to hepatitis experience (41.18%) p-0.0003, Figure 8B.

Reduction in haemoglobin levels was observed for 32.11% of the population, 29.35% for the hepatitis negatives and 47.06% of the hepatitis co-infected. Cluster distributions were comparable among the two groups Figure 8C.

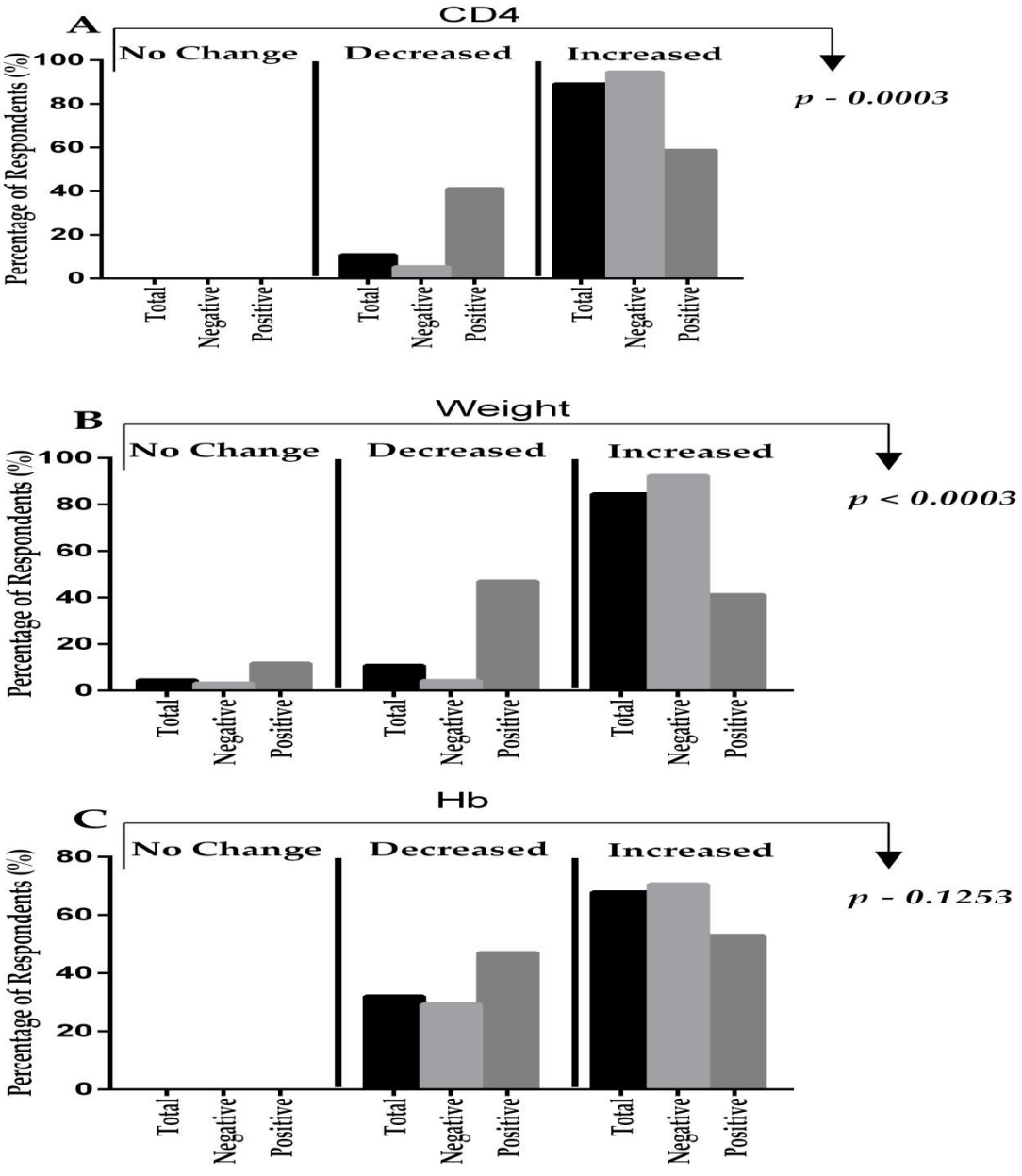


**Figure 8: Respondents twelve months' treatment response clusters stratified by hepatitis viral co-infection. A-CD4, B- Weight and C-Haemoglobin**

All study participants were on the recommended triple therapy regimen of 2 Nucleoside/tide Reverse Transcriptase Inhibitors (NRTIs) and 1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) as depicted in appendix 7. Zidovudine was replaced for Tenofovir in patients who presented with severe anaemia. Unfortunately, recommendations for the treatment of hepatitis co-infection were not adhered to since patients' status on hepatitis co-infection was unknown to the healthcare providers.

# CHAPTER FIVE

# DISCUSSION

## 5.1 INTRODUCTION

The impact of co-infections of HBV and HCV among HIV seropositive patients has become a public health concern. There is reduced survival and an increase risk of disease progression among HIV and hepatitis co-infected patients, compared with HIV monoinfected on therapy (Mohammadi *et al*, 2009). This study attempted to compare the treatment outcome of HIV patients' co-infection with viral hepatitis and HIV alone. This was crucial in order to help sustain the positive achievements of HAART since co-infection with viral hepatitis could compromise the prognosis of HIV treatment.

## 5.2 CHARACTERISTICS OF STUDY PARTICIPANTS

The preponderance of the female gender (70%) may be a reflection of the fact that women may be more sensitive to changes in their health and probably socioculturally inured to seek health care earlier than their male counterparts.

Co-infection among our study participants was commoner among married people (9.52%), indicating heterosexual transmission in our environment as previously documented (Alter, 2003). It would have been anticipated, even though debatable, that singles (divorcees and the

unmarried) would be more exposed to co-infection due to their increased risk of multiple partners.

The high percentage viral hepatitis co-infection among respondents without any formal education and those who were not gainfully employed at the time of the study generally could be due to the low level of awareness that may be associated with illiteracy and therefore behaviours and practices that may increase hepatitis transmission were common and their vulnerability in the society due to their low economic status respectively.

There was also a higher percentage co-infection among respondents who have ever had blood transfusion and shared sharps with others since they constitute efficient means to co-infection in the population (Hauri *et al*, 2004).

## 5.3 PREVALENCE OF VIRAL HEPATITIS CO-INFECTION AMONG STUDY PARTICIPANTS

The overall viral hepatitis prevalence (8.5%) among the study cohort was high. HBV and HCV co-infections were 7.0% and 1.5% respectively. This observation in HBV rate was well corroborated among people living with HIV (PLHIV) attending antiretroviral clinics in rural and urban sites in the Eastern region of Ghana by Kye-Duodu (2013) with a prevalence of 8.8% (28/320), which is in keeping with other investigators across Africa (Puoti *et al*, 2008; Harania *et al*, 2008; Hamza *et al*, 2013) but slightly lower than values obtained in similar

studies in other parts of Ghana (Geretti et al, 2010; Sagoe *et al*, 2012). Co-infections among this group of people is known with variations worldwide which is attributable to differences in geographic regions even within the same country, types of risk groups and the means of exposures involved (Alter, 2002; Lesi *et al,* 2007; Otegbayo *et al*, 2008).

Co-infection with HCV (1.5%) compare favourably with previous works in similar populations with 1.6% in Senegal (Diop-Ndlaye *et al*, 2008), 1.1% in Kenya (Harania *et al*, 2008), 2.2% in Zambia (Kapembwa *et al*, 2011) and 1.6% in the western part of Nigeria (Hamza *et al*, 2013). The low prevalence of HCV may possibly be linked to the very few transfusions among the participants and the non-existence of Intravenous Drug Users (IVDU). These are the major risk factors identified in the transmission of HCV infection among HIV patients. Most medical laboratories now screen for viral hepatitis before blood is issued out for transfusion and the fact that IVDU is a western lifestyle and therefore alien to our region. This finding further suggests sexual intercourse as the route of infection in our society. However, no patient had triple infection which was found to be more or less consistent with findings from other studies in Ethiopia and Nigeria (Otegbayo *et al*, 2008; Wondimeneh *et al*, 2013; Chiekulie *et al*, 2013).

## 5.4 IMMUNOLOGICAL RESPONSE TO ANTI-RETROVIRAL THERAPY AMONG STUDY PARTICIPANTS

The median CD4 levels observed in this study at baseline (drug-naive) was 130.59±3.35 cells/mm3 (HIV mono-infected), 146.22±2.73cells/mm3 (co-infected with HBV) and 186.90±2.2 cells/mm3 (co-infected with HCV) for the various categories of patients which appears immunologically similar, as evidenced by the similar CD4 counts in HIV mono-infected and HIV co-infected at the commencement of treatment as observed in Nigeria (Otegbayo *et al*, 2011). At month six, the mono-infected HIV patients witnessed a significant (p, <0.0001) rise of CD4 to 250.21±1.8 cells/mm3 whilst HIV/HBV and HIV/HCV recorded 142.89±2.17 cells/mm3 and 189.93±2.05 cells/mm3 respectively. There was a further statistically significant increase to 333.73±1.66 cells/mm3 (p, <0.0001) in the HIV alone group as against 172.07±1.88 cells/mm3 in HIV/HBV and 232.27±1.78 cells/mm3 in HIV/HCV groups at month twelve. This observation showed a much improved immune reconstitution based on the drug intake over the period among the mono-infected HIV patients.

Patients co-infected with viral hepatitis showed non-significant (p, >0.05) increase in CD4 over the 12 month period indicating a poor immunological recovery. HIV/HCV (p, 0.9154) co-infected patients however exhibited a much slower response as compared to HIV/HBV (p, 0.8079) group which could be attributable to differences in viral factors where HBV is a DNA virus while HCV is an RNA virus. The findings of this study is consistent with van Griensven and colleagues (2014), who reported worse HAART outcomes for HBV and HCV patients co-

infected with HIV. They discovered that CD4 recovery was lower in both HBV and HCV co-infected patients. Similar studies across Africa (Wondimeneh *et al*, 2013; Taye *et a*l, 2013) also revealed negative immune recovery among co-infected patients compared to HIV alone. However, these results are at variance with that of Sagoe *et al*, (2012) where they disclosed in a related study in Ghana that, HIV co-infection with HBV and HCV did not affect CD4 cell count but reported a worsen immunosuppression among HIV/HBV co-infected participants who tested positive to HBeAg.

There was a fair distribution of CD4 cell count among the HIV mono-infected and the co-infected groups at baseline. However, at the end of the twelve month, all the 17 hepatitis co-infected patients on treatment still had their CD4 cell counts below 500 cells/mm3 with the majority (52.94%) below 200 cells/mm3. It is however suggested that the combined effect of HIV with HBV or HCV resulted in the marginal and non-significant increase in the immune cell (CD4) count with its consequential threat to recovery among HIV infected individuals. On the other hand, the HIV mono-infected group experienced a statistically significant (p, 0.0006) improvement across the various categories with the majority (58.06%) within the 200-500 cells/mm3 bracket and 26.88% crossing the 500 cells/mm3 mark. This is reflective of a positive immunological outcome to therapy and corroborated an evaluation report on HIV mono-infected patients initiating ART in Ghana (Ohene *et al*, 2013).

## 5.5 CHANGES IN LIVER ENZYMES FOLLOWING THE START OF ANTI-RETROVIRAL THERAPY

Liver hepatotoxicity indicative by increase in liver enzymes (ALT and AST) was significantly higher especially among the HIV/HCV co-infected participants at the end of months six and twelve time points of HAART therapy even with no observable difference at the initiation of treatment. Studies elsewhere (Kalyesubula *et al,* 2011; van Griensven *et al,* 2014) also experienced similar trends of sustained increase in liver enzymes up to 12 months after start of therapy in the same group. This observation could be so since the hepatitis status of the patients were not known by the healthcare providers and therefore no decision on the appropriate choice of treatment by avoiding drugs with toxic effect on the liver of patients who were hepatitis co-infected; and the possible inclusion of drugs that are targeted at the treatment of HCV and HBV. This obviously could cause serious derangement of the liver function among these groups of patients.

## 5.6 CLINICAL RESPONSE TO THERAPY AMONG STUDY GROUPS

The study also revealed a significant rise (p, 0.0018) in weight, a useful clinical monitoring tool, among HIV alone participants in the 12 month study duration with a good correlation with increase in CD4 count. This observation was similarly demonstrated by the findings of Ohene *et al,* (2013) in Ghana with a very significant increase in weight over the same period

after initiating therapy. Contrarily, the weight among hepatitis co-infected HIV participants witnessed no significant (p, 0.7417) improvement after the 12 months period.

## 5.7 HAEMOGLOBIN VARIATIONS AMONG STUDY PARTICIPANTS

Also, the investigation demonstrated a significantly lower haemoglobin level among the hepatitis co-infected participants as compared to those with HIV alone. Even though the haemoglobin levels were generally low across the groups, the finding compares favourably with reports that HIV disease impacts adversely on haematological profile of patients due to the enormous assault of the virus on haemopoietic cells/system and worsened when co-infected with viral hepatitis (Opie, 2012). It was also speculated that the resultant low effect on hepatitis co-infection could be due to an additive or synergistic effect of the two infections. Although there are several of possible causes of anaemia in HIV infections, it is commonly linked to bone marrow failure, peripheral destruction and opportunistic infections and HAART therapy.

## 5.8 LIMITATIONS OF STUDY

This study was not without limitations. First, in the number of participants that were enrolled, as more subjects would yield more reliable outcomes. For instance, the hepatitis C co-infected patients were few and could lead to statistical artefacts. Second, it would have been useful to confirm the presence of HBV by HBV DNA and HCV using HCV RNA rather than

serological status alone. Thirdly, scarce resources could not allow the determination of HIV viral load of the participants to help determine the virological response of the various categories of patients. The above notwithstanding, the results were consistent with other studies elsewhere, and therefore very relevant for improving care among HIV/AIDS patients on therapy.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

The study confirms hepatitis as a co-morbid infection and poses a serious threat to HIV positive patients. The overall prevalence of HIV/hepatitis co-infection seen in the Volta region of Ghana is high (8.5%) and should be taken into account by healthcare providers at ART centres. The findings in this study underscore the need to determine hepatitis status of all HIV patients undergoing HAART.

The present study has also shown that immunological and clinical response to treatment among HBV/HCV co-infected HIV positive patients was poorer as compared with the mono-infected patients. Majority of the hepatitis co-infected patients showed an impaired CD4 recovery to therapy and remained at an advanced HIV stage with immunosuppression at the end of the twelve months of study.

Liver hepatotoxicity indicative by liver enzymes was found to be significantly higher in the HCV co-infected participants after commencement of treatment. Meanwhile, the protocols available for HIV management in Ghana are silent on this category of patients may be due to the low prevalence reported in few previous studies in the country.

## 6.2 RECOMMENDATIONS

On the basis of existing literature and the findings of this study, it is recommended that:

- Similar studies are replicated in other regions to accurately determine region specific hepatitis co-infection among HIV patients attending ART centres.
- The National Aids Control Program should enforce hepatitis screening policy among HIV positive patients undergoing HAART by equipping all treatment centres with the necessary resources and logistics. This is important as the choice of antiretroviral and other drugs with potent effect on hepatitis could improve the response to treatment of the affected patients.
- HIV patients co-infected with hepatitis should be closely monitored for liver enzymes following the commencement of therapy.
- Education and general preventive measures on the acquisition of viral hepatitis among HIV patients should be highlighted and the vaccination of those who test negative to hepatitis B virus should be encouraged.

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# APPENDICES

## APPENDIX 1 – PRINCIPLE FOR FIRST RESPONSE® HIV 1-2-0 TEST

First response HIV 1-2-0 test is an immune-chromatographic (rapid) test for the qualitative detection of antibodies of all isotypes (Ig G, Ig M, Ig A) specific for HIV – 1 including subtype O and HIV – 2 in human serums, plasma or whole blood. First Response® HIV 1-2.O Card Test is based on the principle of immune-chromatography in which nitrocellulose membrane is pre-coated with recombinant HIV-1 capture antigens (gp41 including Group O and p24) on test band "1" region and with recombinant HIV-2 capture antigen (gp36) on test band "2" region. This conjugated antigen-antibody complex moves through the nitrocellulose membrane and binds to the corresponding immobilised HIV 1 antigens and HIV 2 antigen (Test Lines) leading to the formation of a colour visible line as the capture antigen-antibody conjugated antigen complex, indicating reactive results.

## APPENDIX 2- PRINCIPLE FOR ORAQUICK HIV 1&2 TEST

The Oraquick HIV type 1 (HIV – 1) rapid antibody test is a rapid test that uses serum, plasma, whole blood and oral mucosa transudate. Oraquick detects gp41 immunodonminant domain antibody.

## APPENDIX 3 - SAMPLING PROCEDURE

1. A sterile, dry 10ml plastic syringe was selected and attached to an appropriate needle.

2. A tourniquet was tied on the upper arm of the patient and was asked to make a fist.

3. Cotton wool soaked in 70% alcohol was used to clean (sterilize) the skin for venipuncture.

4. Venipuncture was made with the bevel of the needle appropriately angled.

5. About 10mls of blood was collected before tourniquet was removed.

6. The needle was removed carefully and the puncture site pressed with a piece of cotton wool to stop bleeding.

7. The needle from the syringe was capped and carefully disposed into the sharps/infectious waste container.

8. The various tubes were filled with the required volume of blood and mixed slowly.

9. The site of venipuncture was inspected for bleeding. A piece of cotton wool was placed on the site and a plaster placed on it.

## APPENDIX 4 - PRINCIPLE OF THE FLOW CYTOMETRY

Individual cells stained with fluorescent labels or absorption dyes are suspended in physiological solution and introduced under a slight pressure through a flow chamber into the centre of a stream of cell-free sheath fluid. The light scattered by the individual particle and the fluorescence emitted by the cells is used for analysis and sorting of the cells based on the fluorescent antibody directed against a specific surface. This combination of scattered and fluorescence light is picked up by the detectors in the flow cytometer. These detectors then produce electronic signals that are proportional to the optical signals received. The visible light undergoes deflection based on the size and internal structures of the cell. FSC (Forward Scatter) correlates with the cell volume. SSC (Side Scatter) depends on the inner complexity

of the particle (i.e. shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). The fluorescence emitted by the cell depends upon the florescence tagged specific monoclonal antibodies against the cell surface markers. The data collected on each cell or event is stored in the computer. This data is then processed and analyzed to provide information about cell populations within the sample.

## APPENDIX 5 – ETHICAL APPROVAL

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
**COLLEGE OF HEALTH SCIENCES**
**SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL**
***COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS***

Our Ref: CHRPE/AP/012/14

29th January, 2014.

Mr. Daniel Adigbli
Ghana Health Service
Post Office Box KK 29
Kete Krachi
Volta Region.

Dear Sir,

LETTER OF APPROVAL

*Protocol Title:* *"HIV, HBV and HCV Co-Infected Patients Response to Highly Active Antiretroviral Therapy in the Volta Region of Ghana."*

*Proposed Site:* *Krachi West District Hospital and Volta Regional Hospital, Ho.*

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 16th December, 2013 from the Krachi West District Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A notification letter of 19th December, 2013 from the Volta Regional Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Acheampong MD, FWACP
Chairman

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Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

**APPENDIX 6 -CONSENT FORM AND QUESTIONNAIRE**

**Statement of person obtaining informed consent:**

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

**Statement of person giving consent:**

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

**Statement of person witnessing consent process (for non-literate participants).**

I_____ (name of witness) certify that information given to _____ (name of participant) in the local language is a true reflection of what I have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE: _____

WITNESS' NAME: _____

**RESEARCH QUESTIONNAIRE FOR HIV-1 POSITIVE CLIENTS**

**SECTION A: DEMOGRAPHIC CHARACTERISTICS OF THE HIV-1 PATIENT**

***Kindly provide straight to the point answers, exact figures and tick brackets where appropriate***

1. How old are you (in years)? .....

2. Gender Male [ ] Female [ ]

3. Which ethnic group do you belong to?

Ewe [ ] Akan [ ] Ga-Adangme [ ] Guan [ ] Mole-Dagbon [ ] Others, [ ] specify……

4. What is your marital status? Single (Never married) [ ] Married [ ]

Divorced [ ] Widowed [ ] Separated [ ] Boy/ Girl Friend [ ]

5. What is your religion? Christian [ ] Islam [ ] Traditionalist [ ] others [ ] (specify)…….

6. What is your occupation? Farming [ ] Trading [ ] Civil Servant [ ]

   Schooling [ ] Apprenticeship [ ] Artisan [ ] Unemployed [ ]

7. What is your highest level of education?

   No education [ ] Primary [ ] Junior High School [ ]

   Senior High School [ ] Vocational [ ] Tertiary [ ]

8. What is your economic/ income status? Low income [ ] Middle income [ ]

   High income [ ]

9. What type of residence/locality do you currently occupy? [ ] Urban [ ] Rural

**SECTION B**

**RISK FACTORS FOR CO-INFECTIONS WITH HEPATITIS B AND/OR HEPATITIS C VIRUSES AND EMMOTIONAL ASSESMENT**

***Kindly choose YES or NO and tick the appropriate brackets throughout this section***

1. Have you received transfusion of blood and blood products in the past? Yes [ ] No [ ]
2. Have you ever had an unprotected sexual intercourse with the opposite sex? Yes[ ] No [ ]
3. Do you share sharps (e.g. needles, blades, etc) with people? Yes [ ] No [ ]
4. Has your mother been diagnosed of having viral hepatitis? Yes [ ] No [ ]
5. Do you inject drugs in groups (intravenous drug user - IDU)? Yes [ ] No [ ]
6. If a man, do you have sex with a fellow man (MSM)? Yes [ ] No [ ]
7. Are you a commercial sex worker (prostitute)? Yes [ ] No [ ]
8. Are you exposed to blood in the course of work (occupational injury)? Yes [ ] No [ ]
9. Are you suffering any form of stigmatization and discrimination as a result of your status? Yes [ ] No [ ]
10. Do you receive social support from family, friends, organizations etc? Yes [ ] No [ ]
11. Have you received counseling on drug adherence? Yes [] No [ ]

## APPENDIX 7 – FIRST LINE DRUGS

| | Drugs | Contra – indications | Comments |
|---|---|---|---|
| First Choice drug | FIRST OPTION<br>Zidovudine + Lamivudine + Nevirapine | Zidovudine is contra- indicated in severe anaemia | Replace with Tenofovir |
| | SECOND OPTION<br>Zidovudine + Lamivudine + Efavirenz | | |
| Second Choice of drug | FIRST OPTION<br>Tenofovir + Lamivudine + Nevirapine | | Tenofovir was used when Zidovudine was contra-indicated (Hb less than 8.0 g/dl) |
| | SECOND OPTION<br>Tenofovir + Lamivudine + Efavirenz | | Tenofovir was used when Zidovudine was contra-indicated e.g. in severe anaemia |