KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF MOLECULAR MEDICINE

THE PREVALENCE OF HEPATITIS B AND/OR HEPATITIS C VIRUS (HBV AND/OR HCV) CO-INFECTION AMONG HIV-INFECTED PREGNANT WOMEN

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN IMMUNOLOGY

BY

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This work has not been submitted for any other degree.

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ABSTRACT

Background

Hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection(s) among people living with human immunodeficiency virus (HIV) is a growing problem. Liver disease resulting from hepatitis B or hepatitis C virus infection has become one of the main causes of morbidity and mortality among persons living with HIV worldwide. Co-infections are known to increase the risk of vertical transmission of these viruses. Various research works have provided varying information on the prevalence and risk factors for HBV and/or HCV co-infection among pregnant women. Very little information on this subject exists in Ghana.

Aims

This study sought to determine the prevalence and risk factors associated with HBV and/or HCV co-infection among HIV infected pregnant women. It also sought to investigate the immunological and virological characteristics associated with the co-infection and the use of antiretroviral drugs among the HIV-infected participants.

Methods

This study was conducted at the antiretroviral therapy and antenatal clinics of the St. Elizabeth Hospital, Hwidiem and the Holy Family Hospital Techiman in the Brong-Ahafo Region of Ghana between May 2012 and May 2013. A total of 124 participants including 74 HIV-infected, and 50 HIV-negative, pregnant women were enrolled. The full blood count and the CD4 count of eligible participants were determined using Sysmex KX N21 haematology analyzer (Japan) and BD Facs Count analyzer (USA) respectively. The hepatitis B profile and anti-HCV status of participants were determined using Wondfo Biotech test-kits. The HIV-I viral load of the HIV positive participants was determined close to the time of delivery using COBAS® AmpliPrep/COBAS® TaqMan Analyzer (USA).

Results

The prevalence of HBV infection among the HIV-infected participants was higher than observed among the HIV-negative participants (14.9% vrs 10%). However, the prevalence of antibodies to the hepatitis C virus (anti-HCV) was lower among the HIV-infected pregnant women as compared to their HIV-negative counterparts (4.1% vrs 12%). None of the participants had a triple infection of HIV/HBV/HCV. There was no significant difference between the co-infection status and the baseline CD4 count as well as the HIV-1 viral load close to the time of delivery. However, persons who were diagnosed of HIV-infection in the third trimester of pregnancy were significantly at risk of having high HIV viral load close to the time of delivery ($p= 0.009$). HBeAg positivity was associated with severe immunosuppression (mean CD4=185± 10). HIV/HBV co-infected participants were significantly less likely to seroconvert from HBeAg to anti-HBe ($p= 0.003$).

Conclusion

HIV/HBV and HIV/HCV co-infections are high among HIV-infected pregnant women. Making ARVs available alone may not lead to the desired results. Priority must be given to early diagnosis and initiation of ARV prophylaxis for HIV infected pregnant women.

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LIST OF ABBBREVIATIONS AND ACRONYMS

ANC	Antenatal Clinic
AIDS	Acquired Immune Deficiency Virus
ART	Anti-Retroviral Therapy
ARVs	Anti-Retrovirals
Anti-HCV	Antibodies to Hepatitis C Virus
Anti-HBs	Antibodies to Hepatitis B surface Antigen
Anti-HBc	Antibodies to Hepatitis B core Antigen
Anti-HBe	Antibodies to Hepatitis B e Antigen
AZT	Zidovudine
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
CTL	Cytotoxic T-Lymphocytes
DNA	Deoxyribonucleic Acid
EFV	Efavirenz
FBC	Full Blood Count
FTC	Emtricitabine
HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HBeAg	Hepatitis B E Antigen
HBsAg	Hepatitis B surface Antigen
HBcAb	Antibodies to Hepatitis B core Antigen

HSC	Hepatic Stellate cells
IgM	Immunoglobulin M
IgG	Immunoglobulin G
MTCT	Mother to Child Transmission
MCP	Monocyte Chemoattractant Protein-1
NVP	Nevirapine
NK cells	Natural Killer Cells
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
OD	Optical Density
PMTCT	Prevention of Mother to Child Transmission
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
STD	Sexually Transmitted Disease
SVR	Sustained Virological Response
TDF	Tenofovir Disoproxil Fumarate
TLR	Toll-like receptors
TNF	Tissue Necrosis Factor
TRIAL	Tumour Necrosis Factor –Related Apoptosis Inducing Ligands
UNAIDS	Joints United Nations Programme on HIV/AIDS
WBC	White Blood Cells
3TC	Lamivudine

CHAPTER ONE

INTRODUCTION

1.1 Background

The approximated number of people living with human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) worldwide in 2010 was 34 million. Sub-Saharan Africa bears the heaviest burden with 68% of all persons infected with HIV (UNAIDS 2011).

The estimated adult national HIV prevalence in Ghana was 1.5%, in the year 2011. In that same year, an estimated 225,478 persons (100,336 males and 125,141 females) were living with HIV and AIDS. There were 12,077 new infections and 15,263 AIDS deaths. The number of children who lived with HIV in 2011 was 30,395. A total of 1,704 new child infections occurred in almost equal proportions by gender. The annual AIDS deaths in children were estimated at 2,080 (NACP, 2012b).

The median HIV prevalence, recorded among pregnant women was 2.1 percent in 2011 with sentinel site prevalence ranging from 0.0 percent in Adibo to 9.6 percent in Cape coast (NACP, 2012a).

The approximated worldwide carriers of hepatitis B virus are 350 million (Lok and McMahon, 2009), with an estimated 50 million chronic carriers of HBV in Africa. In sub-Saharan Africa, carrier rates range from 9% to 20%. HBV is endemic in Ghana with sero-prevalence rates ranging from 6.7% to 10% in blood donors, 6.4% in pregnant women and 15.6% in children among the general population (Blankson et al, 2005).

It is estimated that over 170 million people are infected with HCV worldwide (WHO, 2012). The prevalence of healthy carriers with antibodies to HCV in Africa varies from 0.5% to 10%, even though it may exceed 20% in some cases (Blankson et al, 2005).

HCV is a major causative agent of liver related morbidity and mortality worldwide (Rotman and Liang, 2009). It is an important cause of community acquired non-A and non-B hepatitis in certain parts of Africa. Recent studies have revealed HCV sero-prevalence rates of 2.8% to 5.4% in Ghana (Blankson et al, 2005).

HIV, HBV and HCV epidemics overlap because they share similar routes of transmission (den Brinker et al, 2000; Wondimeneh et al, 2013),with around 10% of the HIV-infected population estimated to have chronic HBV infection and around 33% of the HIV-infected population estimated to have chronic HCV infection (Landes et al, 2008).

Co-infection of the HIV infected pregnant women with the hepatitis B or hepatitis C viruses is a growing problem. HIV, HBV and HCV infections are of immense concern because of their prolonged viraemia and carrier or latent state. They also cause fatal, chronic and life-threatening conditions (Landes et al, 2008).

Viral hepatitis during pregnancy is associated with high risk of maternal complications and has a high rate of vertical transmission causing foetal and neonatal hepatitis (Elsheikh et al, 2007).

Without intervention up to 40% of HIV positive mothers transmit HIV to their babies (UNAIDS, 2012b).

The clinical management of HIV-infected individuals co-infected with HCV or HBV is challenging (Wondimeneh et al, 2013), and HIV infection is known to have a negative impact on the outcome of HCV and HBV infections (Lincoln et al, 2003; Wondimeneh et al, 2013) . Coinfection with hepatitis B virus or hepatitis C virus adversely affects the prognosis of HIV infection and results in complex interactions with antiretroviral therapy (Nyirenda et al, 2008). Few studies have addressed the issue of co-infection with HCV and/or HBV in HIV-infected pregnant women to date (Landes et al, 2008).

This study is therefore very necessary to ascertain the issue of co-infection of these viruses in pregnant women in other to deal more effectively with their vertical transmission.

1.2 Problem Statement

Highly active antiretroviral therapy (HAART) has improved the health of people living with human immunodeficiency virus (HIV), acquired immune deficiency syndrome (AIDS)-related morbidity and mortality have decreased whereas mortality from hepatitis and liver disease, has increased (Lincoln et al, 2003; Rockstroh et al, 2008; Wondimeneh et al, 2013). Among HIV-infected individuals, chronic co-infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) are associated with excess morbidity and mortality (Buskin et al, 2011). Liver disease, from HBV/HCV or other causes, is now one of the most common causes of death among HIV-infected people (Buskin et al, 2011). Therefore, this study seeks to determine the prevalence of HBV and HCV among HIV-infected pregnant women and investigate their immunological and virological characteristics and the impact of antiretroviral therapy.

1.3 Justification

In sub-Saharan Africa, where HBV is endemic, 13% of HIV-infected pregnant women also have HBV (Jonas, 2009).

Several studies in other countries have shown a higher prevalence of hepatitis B or hepatitis C co-infections among HIV infected pregnant women than in the HIV negative pregnant women. Ghana has different cultures, geographical location, genetics, and economic status from these countries.

Several studies have been done on HIV, HCV and HBV infections in Ghana (Gerretti et al, 2010; Sagoe et al, 2012).

However, there are no data on the prevalence of HIV/HCV or HIV/HBV co-infection in antenatal populations in the country.

In Ghana, little attention is given to HCV and HBV screening among HIV infected persons. Even when HCV and/or HBV screening is carried out among people living with HIV, there are no clear recommendations concerning the frequency of repeat screening following a negative test for HBV and HCV when risk of infection remains present.

It is important to follow trends in HBV and HCV infection among people living with HIV for care planning purposes, prevention, education, and treatment of preventable and modifiable factors contributing to liver disease (Buskin et al, 2011). Hence this study seeks to ascertain the prevalence of HCV and/or HBV co-infections among HIV-infected pregnant women and to investigate their immunological characteristics, virological characteristics and the impact antiretroviral therapy.

1.4 Research Questions

- What is the prevalence of HBV and HCV among HIV infected pregnant women
- What is the effect of HIV/HBV and HIV/HCV co-infections on HIV-1 RNA viral load of the HIV-infected pregnant women close to the time of delivery
- What are the possible risk factors associated with the acquisition of HIV/HBV and HIV/HCV co-infection among the study group

1.5 General Objective

To determine the prevalence of hepatitis C or hepatitis B virus (HCV or HBV) co-infection among HIV-infected pregnant women, and to investigate their immunological and virological characteristics and antiretroviral therapy use.

1.6 Specific Objectives

- To determine the prevalence of hepatitis C or B virus (HCV or HBV) co-infection among HIV-infected pregnant women.
- To determine the effect of HIV/HBV and HIV/HCV co-infections on the HIV-viral load close to the time of delivery.
- To determine the risk factors that may be associated with acquisition of co-infection in the study population and the impact of antiretroviral therapy

1.7 Study Hypothesis

Co-infections of HCV and /or HBV in HIV-infected pregnant women may lead to detectable HIV-viral load at delivery and therefore increasing the risk of mother to child transmission of the HIV

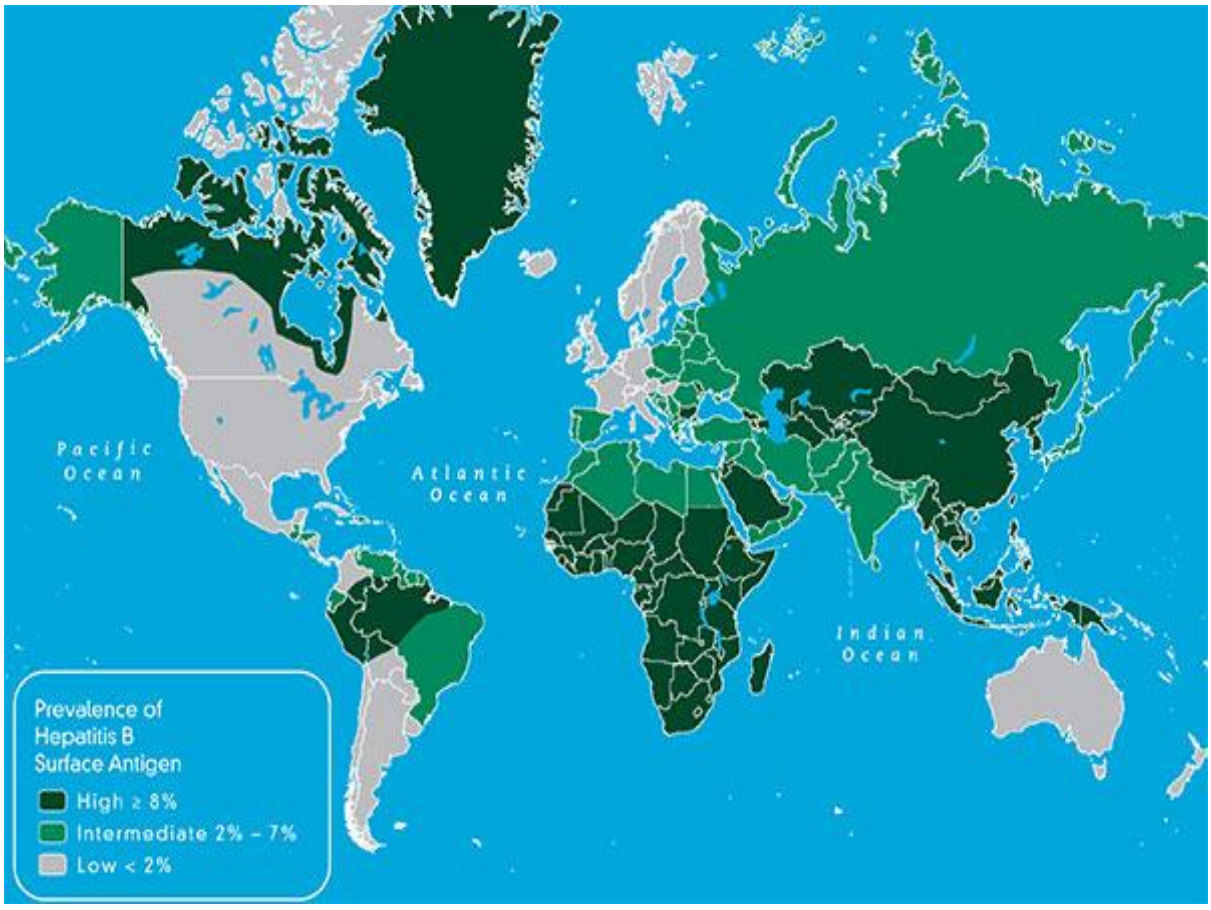
CHAPTER TWO

LITERATURE

2.1 Epidemiology of HIV, HBV and HCV Infections

2.1.1 Epidemiology of HBV infection

The world can be divided into areas of high HBV endemicity, intermediate HBV endemicity and areas of low HBV endemicity (Hou et al, 2005). About 5% of the world's population are chronically infected with HBV (Burnett et al, 2005).



CDC, 2012
FIG 1; Global distribution of chronic HBV infection

Approximately 45% of the global population live in areas with high prevalence of chronic HBV infection (> 8% of the population is HBsAg positive) (Hwang and Cheung, 2011); 43%

in areas with intermediate prevalence (2%-8% of the population is HBsAg positive); and 12% in areas with a low prevalence (< 2% of the population is HBsAg positive) (Burnett et al, 2005).

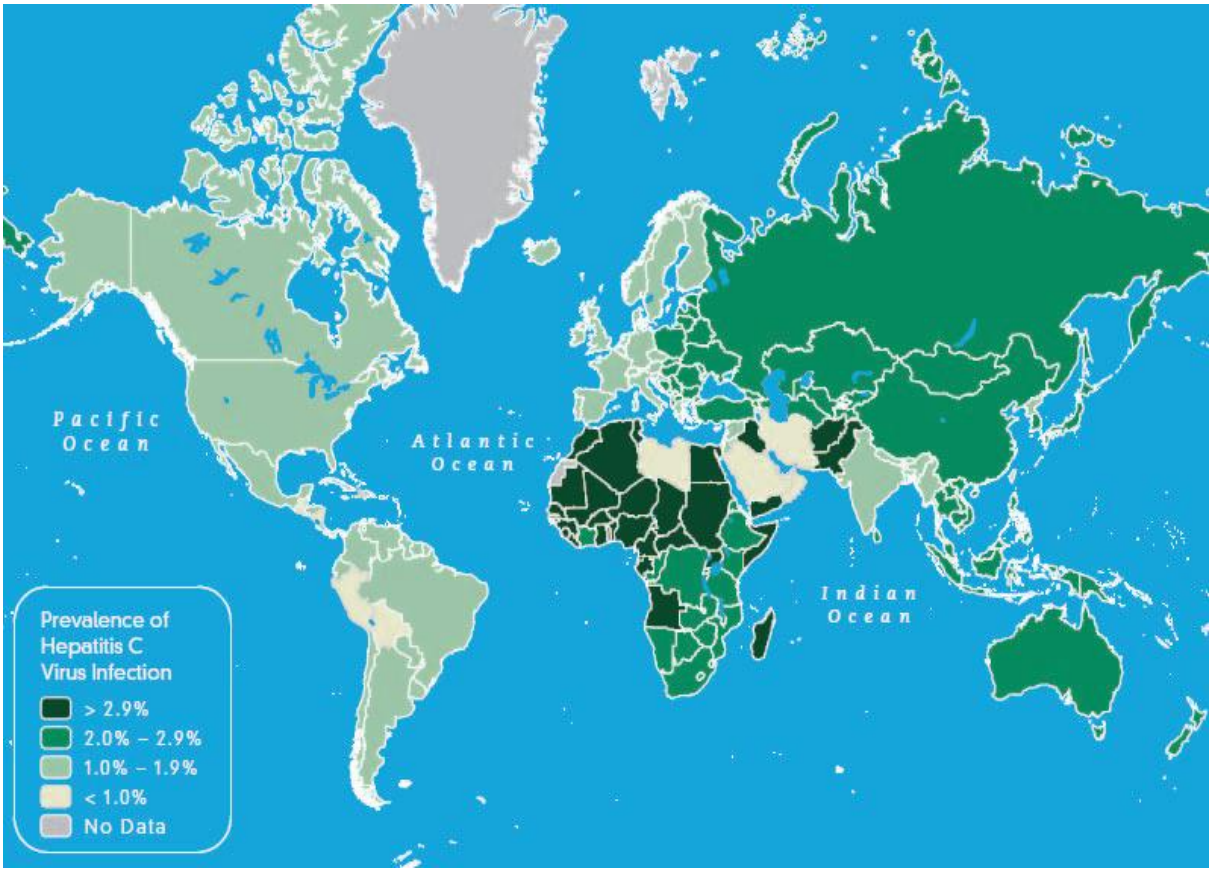
The age at which most infections occur is the primary factor that affects the endemicity of HBV infection (Hou et al, 2005). HBV infection is highly endemic in those parts of the world where most infections occur during the perinatal period or early in childhood, this includes Asia, sub-Saharan Africa (Alter, 2006) and the Western Pacific areas (Burnett et al, 2005). In high prevalence areas, the lifetime risk of HBV infection is > 60% (Hwang and Cheung, 2011) and there is 70%–90% serological evidence of previous HBV infection (Shepard et al, 2006). In these areas, because most infections in children are asymptomatic, very little acute disease related to HBV occur, but the rates of chronic liver disease and liver cancer in adults are very high (CDC, 1995).

In areas of intermediate prevalence, the life time risk of being infected by HBV infection is between 20%-60% and infections occur in all age groups (Burnett et al, 2005). There is 10% to 60% serological evidence of previous HBV infection (Hou et al, 2005). North Africa, Southern and Eastern Europe fall under this category (Burnett et al, 2005). Acute disease related to HBV is common in these areas because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained mostly by infections occurring in infants and children (CDC, 1995).

In low prevalence areas, the lifetime risk of infection is <20% (Burnett et al, 2005). This includes most developed nations for example, Western Europe, Australia and USA. Most infections occur among adult populations with high risk behaviours that include injection drug users, persons with multiple heterosexual partners, and men who have sex with men (Alter, 2006).

2.1.2 Epidemiology of HCV

HCV infection has a worldwide distribution (Lavanchy, 2011). HCV infects at least 3% of the global population and it is a leading global cause of liver disease (Pybus et al, 2007). HCV infects persons of all ages, genders, races and regions of the world (Lavanchy, 2011). Similar to the categorization of the global distribution of HBV infection, the differences in endemicity of HCV infection can be described based on regional prevalence (Alter, 2006). The world can be categorised into areas of high endemicity (prevalence ≥3%), moderate endemicity (prevalence 2–2.9%), low endemicity (prevalence 1.0–1.9%), and very low endemicity (prevalence < 1.0%) (Averhoff et al, 2012).



CDC, 2012
Fig 2; Global distribution of hepatitis C infection

Northern Africa (particularly Egypt), has the highest prevalence of HCV infection (Lavanchy, 2011). Eastern Europe and most of Asia have moderate prevalence of HCV

infection, low prevalence has been reported from Western Europe, North and South America, and Australia, and very low prevalence from Northern Europe and the UK (Alter, 2006).

The predominant mode of transmission in high prevalence and moderate prevalence countries is unsafe therapeutic injections administered by both professionals and non-professionals (Shepard et al, 2005; Alter, 2006). This may account for up to 40% of HCV infections globally (Alter, 2006). In such areas, injections are often given to deliver medications that could have been administered orally. There is inadequate supply of sterile syringes and non-professionals administer injections outside the hospital settings (Shepard et al, 2005).

There is poor attention to proper cleaning of medical equipments in hospitals and dental clinics. Blood for transfusion purposes are not well screened. These may also be important sources of transmission of HCV (Alter, 2006).

In most areas where HCV prevalence is low, illegal injection drug use is the most important mode of transmission (Averhoff et al, 2012). The transmission of HCV through blood transfusion and organ transplant has virtually been eliminated (Shepard et al, 2005).

However, outbreaks of iatrogenic transmission of HCV continue to occur. This is associated with contamination of multiple dose medication vials and intravenous solutions by the reuse of disposable needles and syringes (Alter, 2006).

2.1.3 Epidemiology of HIV infection

Globally, an estimated O.8% of adults aged between 15 and 49 are living with HIV (UNAIDS, 2012a). The proportion of women living with HIV is approximately 50% (WHO, 2013b).

An estimated 17.7 million women lived with HIV worldwide in 2012 (UNAIDS, 2013). The number of children approximated to be living with HIV increased to 3.3million [3.0 million–3.7 million] in 2012(UNAIDS, 2013).

The number of children that died from AIDS related causes reduced by 29% in 2011. This is attributed to the steady extension of services to prevent transmission of HIV to infants and an increase in access to treatment for children (WHO, 2013b).

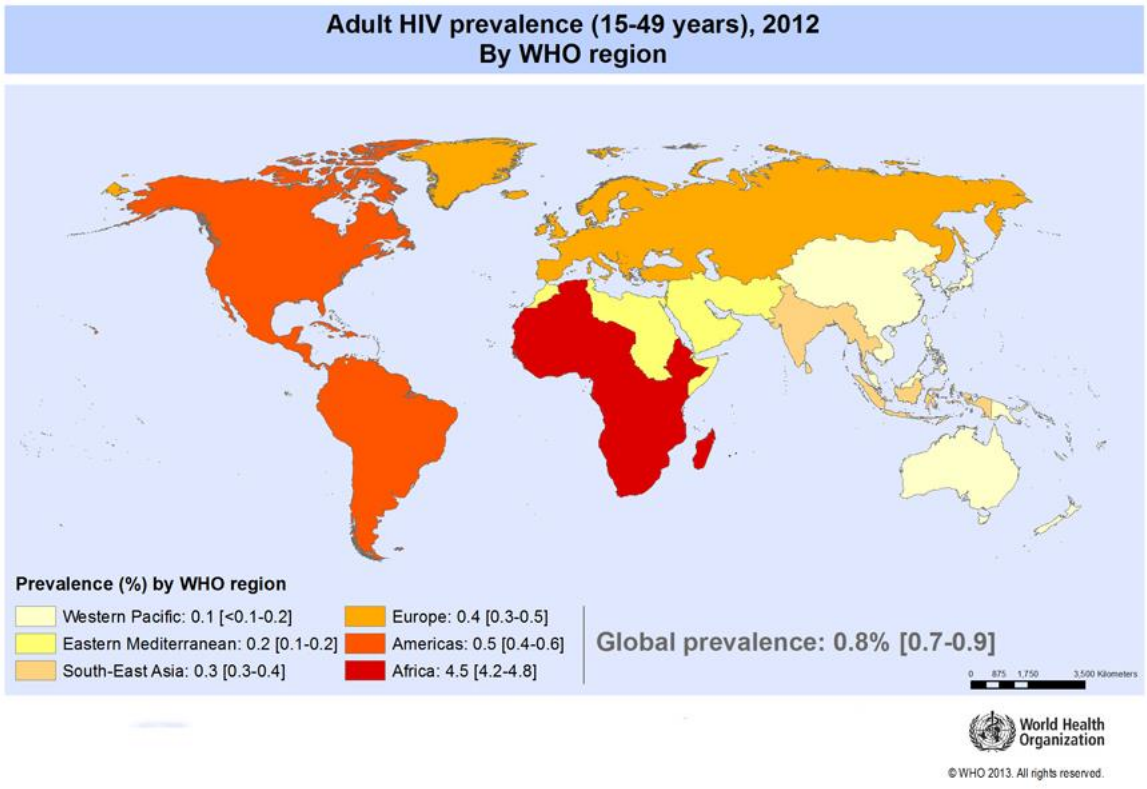


Fig 3; Adult HIV prevalence (aged 15-49) (WHO, 2013a)

The burden of the epidemics varies significantly between regions and countries. The most severely affected part of the world is sub-Saharan Africa where virtually 1 in 20 adults

(4.9%) is living with HIV (UNAIDS, 2012a). Women make up 58% of adults living with HIV in sub-Saharan Africa (WHO, 2013b).

In Eastern Europe, the Caribbean and Central Asia, 1% of the adult population were living with HIV in 2011. This makes these areas the most heavily affected regions after sub-Saharan Africa (UNAIDS, 2012a).

The regional prevalence of HIV in sub-Saharan Africa is almost 25 times higher than in Asia. However it is worth noting that about 5 million people are living in with HIV in South, South-East and East Asia combined (UNAIDS 2012a).

2.1.4 Epidemiology of HCV and/or HBV Co-Infection with HIV

Among the estimated 33.3 million persons infected with HIV worldwide in 2009, about 4 million were chronically infected with HBV and an estimated 5 million were chronically infected with HCV (Lacombe and Rockstroh, 2012). These estimates of co-infection are influenced by several factors, including geographic differences in the prevalence of chronic infection by age, the efficiency of exposures that account for most transmission, and the prevalence of persons at high risk for infection (Alter, 2006).

HIV, HBV and HCV are transmitted through parenteral, sexual and perinatal exposures but they differ in the efficiencies of these routes (Alter, 2006, Rotman and Liang, 2009).

Hetero sexual exposures accounts for most HIV infections in sub-Saharan Africa which has the highest HIV prevalence in the world (Lewis, 2011). HBV infection is highly endemic in sub-Saharan Africa (Kew, 2012) because most HBV infections occur in early childhood or through perinatal transmission routes (Alter, 2006). There is a high prevalence of HBV among adolescents and adults at risk of sexually transmitted HIV because, HBV infection

acquired vertically or in early childhood is more likely to lead to chronic infection (Alter, 2006).

In developed countries the prevalence of HIV is low (Alter, 2006). The prevalence of chronic HBV infection is also low in the developed world (Hou et al, 2005; Shepard et al, 2006) because most native infections occur in adults who are less likely to develop chronic HBV infection (Alter, 2006). Sexual transmission and injection drug use are the most important routes of transmission for both HBV (Hou et al, 2005; Shepard et al, 2006) and HIV in developed countries (Alter, 2006).

HCV is not efficiently transmitted by perinatal or sexual exposures, which are vital modes of transmission for HBV and HIV (Alter, 2006). Parenteral exposure to infected blood is the most predominant mode of transmission of HCV (Shepard et al, 2005). Injection drug use, blood transfusions from unscreened donors, unsafe therapeutic injections, and other health-care related procedures are the risk factors that are most frequently cited for the majority of HCV transmissions worldwide (Shepard et al, 2005).

Differences have been observed between HIV, HBV and HCV infectivity in several environments (Alter, 2006). Exposures to contaminated needle sticks among healthcare workers in the occupational environment, have demonstrated that HCV is 10 times more infectious than HIV, and HBV is 100 times more infectious than HIV (Wasley and Alter, 2000).

Even though HIV has been propagated in cell culture after drying at room temperature, it may not be infectious in humans. However, experimental studies in animals have demonstrated that HBV and HCV can remain viable in dried blood on environmental surfaces at room temperature (Alter, 2006).

HCV and HBV have different environmental survival capabilities compared with HIV. This may be one of the reasons why injection drug users acquire HCV and HBV more rapidly than HIV infection (Alter, 2006).

This may also be one of the explanations for the association of HBV and HCV infections with sharing drug preparation equipment in addition to needles and syringes, and the more frequent episodes of iatrogenic transmission of HBV and HCV (Alter, 2006).

2.2 The Structure and Genetics of HIV, HBV and HCV

2.2.1 The structure and genetics of HIV

HIV belongs to the family retroviridae and the genus lentivirus. The genome of the HIV contains three genes (gag, pol and env) which code for three groups of structural proteins (Fanales-Belasio et al, 2010). The gag gene codes for the proteins: p17, p24 p7 and p9. The products of the pol gene include protease, endonuclease, integrase and reverse transcriptase. The env gene codes for a large protein. This is glycosylated and cleaved to form gp41, the transmembrane protein and gp120 (Simmonds and Peutherer, 2002b). Both ends of the genome have long terminal repeat (LTR) sequences which contain promoter and enhancer sequences (Fanales-Belasio et al, 2010).

HIV has two regulatory genes (tat and rev) and four genes (nef, vif, vpr and vpu) that code for accessory proteins.The HIV-2 does not contain vpu but it rather contains the vpx gene (Hope and Trono, 2000). A protein that has a general stimulating effect on the synthesis of all HIV viral proteins is coded for by the tat gene. The product of the rev gene regulates the viral protein synthesis by favouring the production of full-length RNA molecules rather than the spliced RNA from the regulatory genes (Simmonds and Peutherer, 2002b). Most of the small accessory proteins of HIV have multiple functions (Hope and Trono, 2000).

2.2.2 The structure and genetics of HBV

HBV is a member of the family Hepadnaviridae (Locarnini, 2004) and the genus Orthohepadnavirus (Huang et al, 2006). HBV is the smallest human enveloped DNA virus (Chevaliez and Pawlotsky, 2008). It has a viral DNA which is circular and it is made up of approximately 3,200 base pairs (Simmonds and Peutherer, 2002a). The HBV DNA genome encodes at least four overlapping open reading frames, including the surface (preS/S), precore/core (preC/C), polymerase (P) and X genes (Locarnini, 2004).

Based-on an 8% or more difference in the DNA sequence over the full genome, HBV strains are classified into 8 genotypes (A to H) (Locarnini, 2004). Some HBV genotypes have worldwide distribution whereas others have a more restricted geographical distribution (Simmonds and Peutherer, 2002a). The different HBV genotypes seem to have different biological properties that may affect the clinical outcome of HBV-related disease (Chevaliez and Pawlotsky, 2008).

2.2.3 The structure and genetics of HCV

HCV belongs to the Flaviviridae family and the genus Hepacivirus (Brass et al, 2006). HCV is an enveloped RNA virus, with a single stranded RNA genome of positive (coding) polarity (Morgan-Capner and Simmonds, 2002). The HCV genome is made up of approximately 9,600 nucleotides (Kim and Chang, 2013).

The HCV RNA genome contains a major open reading frame that encodes a large polyprotein of approximately 3,000 amino acids (Chevaliez and Pawlotsky, 2008). The polyprotein is cleaved by proteases during and after translation to yield a number of structural and non-structural proteins (Morgan-Capner and Simmonds, 2002).

There are six (6) major genotypes of HCV (Kim and Chang, 2013). However a seventh one has been recently identified. The HCV genotypes are associated with distinct geographical distributions (Chevaliez and Pawlotsky, 2008).

2.3 Effects of HIV/HCV and HIV/HBV Co-infection on the Natural History of these Viruses

The life cycle and the natural history of HCV are significantly affected by co-infection with HIV (Rotman and Liang, 2009). There is an increased rate of progression to cirrhosis, decompensated liver disease, hepatocellular carcinoma, and death in HIV/HCV co-infected persons (Koziel and Peters, 2007).

There are increased HCV-RNA levels in HIV/HCV co-infected persons. This could be related to acquired immunodeficiency and the direct interactions between the viruses. HIV has been shown to increase the replication of HCV and subgenomic replicons forms in tissue culture in-vitro. This increase is mediated through the interaction of HIV gp120 with CCR5/CXCR4 and is dependent on transforming growth factor β1 (Rotman and Liang, 2009).

HIV/HCV co-infected individuals show small increase in serum levels of HCV-RNAs after the initiation of highly active anti-retroviral therapy. This suggests that immune suppression and the direct effect of HIV are not the only factors involved. Co-infection of these viruses is associated with increased mortality as compared to mono-infection of either virus (Lacombe and Rockstroh, 2012).

HIV/HBV co-infections also accelerate HBV liver disease. This is particularly so when HIV associated immunodeficiency progresses, leading to an increase in morbidity and mortality (Kew, 2012).Anti-retroviral therapy has been demonstrated to slow down the progression of liver fibrosis and to decrease liver disease progression (Lacombe and Rockstroh, 2012). This

may be the reason why early initiation of HIV treatment is recommended for HIV/HBV or HIV/HCV co-infected persons (WHO, 2013b).

Co-infection with HBV may not have a substantial impact on the natural history of HIV, in terms of immunologic or virologic responses to ART and HIV-related death (Luetkemeyer, 2010).

Treatment interruptions in persons undergoing therapy with HIV-HBV dual active drugs has been shown to be associated with greater decrease in CD4 T-cells and a faster treatment re-initiation than in HIV mono-infected persons undergoing treatment interruption (Lacombe and Rockstroh, 2012).

HCV accelerates the progression of HIV disease. Hepatitis C might be considered to be a co-factor for HIV disease progression. In a study co-infected patients who had a baseline CD4 cell count of between 50 and 200cells/mm^3 progressed to death more quickly than their HIV mono-infected counterparts (Soriano et al, 2002). HCV infection is also associated with changes in cognitive and psychiatric function, increased prevalence rate of diabetes mellitus and decreased quality of life. These together with liver disease as a result of HCV infection potentially affect the management of HIV (Koziel and Peters, 2007).

The potential benefit of ART is limited by co-infections of HIV, HBV and HCV. This is because HBV or HCV co-infections with HIV is associated with independently increased risk for hepatotoxicity once anti-retroviral therapy is initiated (Kim et al, 2008).

In the management of co-infected persons, control or better cure of hepatitis remains the most desirable goal (Lacombe and Rockstroh, 2012).

2.4 The Pathogenesis of Liver Disease in HIV-HBV Co-infections

Many possible reasons account for the increased liver related mortality in HIV-HBV co-infection as compared to a mono-infection with either virus. These reasons may come from immune factors, viral factors and hepatic factors (Iser and Lewin, 2008). HIV can cause changes in liver function through direct or indirect mechanisms (Crane et al, 2012).

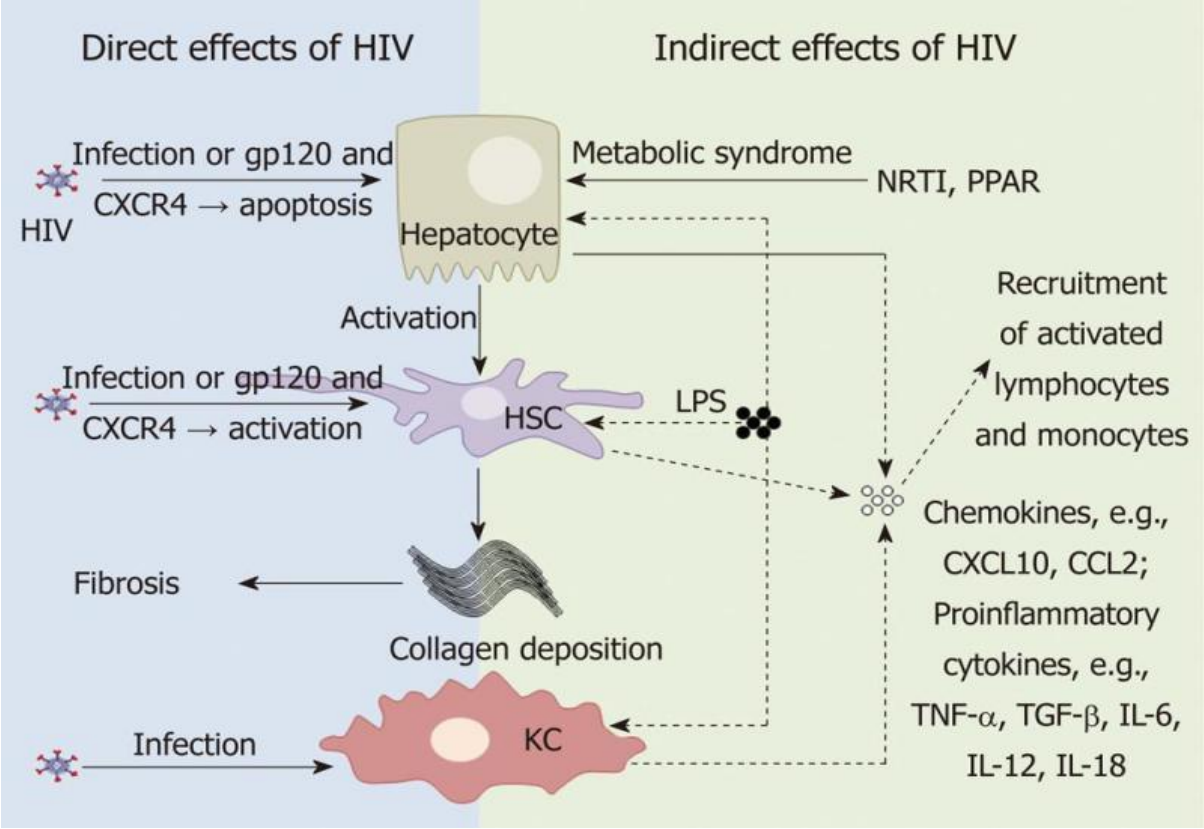


Fig 4; HIV infection and the liver (Crane et al, 2012)

Usually, entry of HIV into target cells is through CD4 receptors together with either co-receptor CXCR4 or CCR5 (Clapham and McKnight , 2001). However, most hepatoma cell lines and primary hepatocytes do not express CD4 (Cao et al, 1990). Hepatoma cell lines may be infected by HIV through receptor mediated endocytosis or alternative co-receptors (Crane et al, 2012). A number of other co-receptors including CCR3, CCR2b, CCR8, Apj, Strl33, Gpr1, Gpr15, CXCR1, ChemR23 or RDC1can also be employed. It is unclear

whether these alternative co-receptors are found on hepatocytes (Iser and Lewin, 2008). It may be possible for hepatocytes to act as transient HIV reservoir. This can facilitate CD4+ T cell infection through cell to cell contact (Crane et al, 2012).

Hepatic stellate cells can be infected directly by HIV. The hepatic stellate cells also support HIV viral gene expression, and are capable of transmitting infectious virus to susceptible lymphocytes through cell to cell contact (Tuyama et al, 2010). When HIV infects hepatic stellate cells (HCS), collagen 1 is expressed. The secretion of the proinflammatory cytokine, monocyte chemoattractant protein 1 (MCP-1) is also induced. These results support direct proinflammatory and profibrogenic effects of HIV on stellate cells (Tuyama et al, 2010). HIV infection of HCS also makes them show increased activation and fibrogenesis. This may explain the increased fibrogenesis seen in HIV-HBV and HIV-HCV co-infections (Crane et al, 2012).

Mutations in the hepatitis B virus can play an important role in the pathogenecity of HBV in HIV-HBV co-infection (Fang et al, 1993). A number of mutations can be observed in HBV mono infection and/or in HBV/HIV co-infection (Audsley et al, 2010). Advanced liver disease and hepatocellular carcinoma can be strongly associated with combinations of these mutations rather than a single mutation (Audsley et al, 2010).

Immune factors necessary for the successful clearance of acute HBV infection involves the innate immune response as well as both the cellular and humoural arms of the adaptive immune response (Bertoletti and Gehring, 2006). However, the mechanism involved is not completely understood (Iser and Lewin, 2008).

The innate immune system is made up of different components. The presence of invading pathogens is recognised by these components. They also provoke quick and effective

production of proinflamatory cytokines and chemokines that limit viral replication and induce maturation of the adaptive immune responses (Bertoletti et al, 2010).

The innate immune response is activated by pathogens through the use of pattern-recognition receptors (PRRs). The PRRs recognise specific structures such as viral genetic material and bacterial cell wall. Toll-like receptors (TLRs) are one of the most important PRRs during viral infections (Busca and Kumar, 2014).

Viral infection of liver cells leads to the release of type-1 interferon (interferon-α/β). This activates natural killer (NK) cells ($CD3^-$ and $CD56^+$) and T-cells ($CD3^+$ and $CD56^+$). The Nk-cells and NK T-cells can remove infected cells. They are also a vital source of interferon- γ and tissue necrosis factor (TNF-α) which are cytokines that inhibit viral replication (Larrubia et al, 2009).

The components of the innate immune response are very important before an effective adaptive immune response develops (Kakimi et al, 2000). Antigen presenting cells (such as Kupffer cells) are recruited by the cytokines during HBV infection. Kupffer cells may induce hepatic injury (Tang et al, 2003). It has however been reported that in HIV-HBV co-infections as well as HIV or HBV mono-infections, there is reduced NK cell mediated cytotoxicity (Iser and Lewin, 2008).

HIV significantly alters the expression and function of Toll-like receptors (TLR), which may be important in immune activation and response to other pathogens, including HBV and HCV (Hernández et al, 2011).

In HIV infected individuals with natural immunity to HBV, there are reduced HBV-specific CTL responses as compared to HIV-negative individuals with resolved HBV infection (Iser and Lewin, 2008). HIV-HBV co-infected individuals have considerably reduced HBV-

specific $CD4^+$ T-cell responses as compared to individuals who are mono-infected with HBV (Chang et al, 2005).

HBV-specific CD8+ T cells play a major role in the control of HBV replication and in the pathogenesis of liver disease when activated. However, its function is impaired during HBV/HIV co-infection. This may explain why acute HBV infections in HIV/HBV co-infected persons frequently develop into chronic HBV infection as compared to HBV mono-infected persons (Lacombe et al, 2010).

Acute HIV infection can lead to a decrease in HBV DNA and HBeAg loss in some individuals with chronic HBV infection. This is possibly due to the release of HIV induced non-cytolytic cytokines (Thio et al, 2004).

Inflammation and fibrosis of hepatic stellate cells and hepatocytes may bring about apoptosis. However, these processes could also be increased by apoptosis (Canbay et al, 2004). The apoptosis of liver cells is fundamental to the process of hepatic inflammation that leads to fibrosis (Iser and Lewin, 2008).

In the setting of HIV infection, there is altered T-cell numbers and reduced CD4/CD8 ratio. This could cause increased hepatic apoptosis. Increased hepatic fibrosis in HIV-HBV co-infection might be attributed to changes in apoptosis but there is no direct evidence in support of this (Iser and Lewin, 2008).

Fibrogenesis mediated by hepatic stellate cells (HSCs) is an important hepatic factor that influences the pathogenesis of liver disease in the setting of HIV-HBV co-infection (Iser and Lewin, 2008). Hepatic stellate cells can go through transformation from a dormant state to a myofibroblast. They are important in the formation of extracellular matrix and collagen when activated (Canbay et al, 2004). HSCs also produce proinflammatory cytokines and

phagocytose apoptotic bodies which are fibrogenic, once activated (Iser and Lewin, 2008). Apoptosis of HSCs can occur spontaneously (this is uncommon in vivo) or through death receptors. HSCs (resident perisinusoidal mesenchymal cell type) are a major effector cells that produce fibrosis in liver injury (Canbay et al, 2004).

In HIV infection, the cytolytic activity and chemokine production of natural killer cells are reduced. The suppression of HSC activity by NK cells may therefore be reduced in HIV-HBV coinfection as compared to HBV infection (Iser and Lewin, 2008).

2.5 The Pathogenesis of Liver Disease in HIV-HCV Co-infections

In HIV/HCV co-infected persons, there is an accelerated liver disease progression (Operskalski and Kovacs, 2011). Liver disease progression typically takes up to 30 years or longer in HCV mono-infected persons. It has however been shown that, liver disease progression in HCV-HIV co-infected person takes less than half the time it takes in the HCV mono-infected persons (Soriano et al, 2002).

HIV-HCV co-infection has significant adverse clinical consequences. HIV infection raises HCV transmission and reduces the rate of spontaneous HCV clearance. This leads to higher rates of chronic HCV infection and it is associated with higher HCV RNA levels (Tuyama et al, 2010).

The mechanism by which liver disease progression is accelerated in HIV-HCV co-infected persons is not well understood. Direct viral effects and immunologic alterations such as immune activation, apoptosis, and diminished HCV specific T-cell response may be involved in the acceleration of liver disease in the setting of HIV-HCV co-infection (Operskalski and Kovacs, 2011).

HIV associated immunosuppression partially contributes to the pathogenesis of chronic liver disease (Pol et al, 2004). It has been shown that, there is a correlation between lower CD4, HCV persistence and liver disease progression (Tuyama et al, 2010).

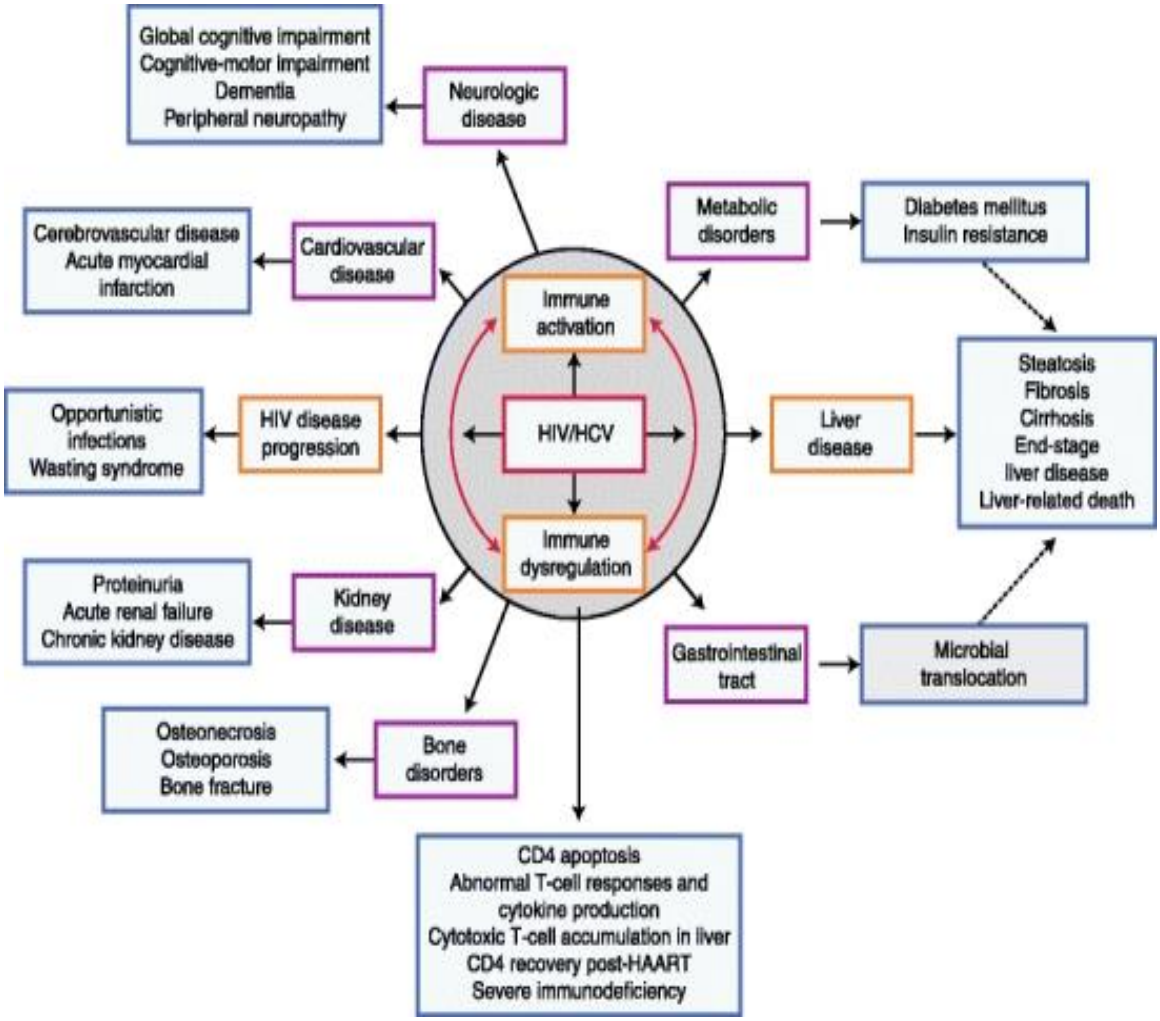


Fig. 5; The pathogenesis of HIV and HCV disease progression in the setting of HCV-HIV co-infection; including immune system dysfunction and clinical complications (Operskalski and Kovacs, 2011).

Accelerated liver disease could also be due to increases in apoptosis of hepatocytes through a Fas/FasL pathway when there is HIV-HCV co-infection (Körner et al, 2011).

Cytotoxic CD8 T-cells accumulates in the liver of persons who are co-infected with HIV and HCV. This brings about increases in inflammatory mediators in co-infected persons as

compared to HCV mono-infected persons and may lead to increased tissue damage in the co-infected patients (Operskalski and Kovacs, 2011).

The infection of activated Hepatic stellate cells by HIV promotes collagen expression and secretion of proinflammatory cytokines.

HIV also induces the apoptosis of hepatocytes causing the release of inflammatory chemokines and cytokines that promote fibrosis. Tumor necrosis factor–related apoptosis—inducing ligand (TRAIL)-mediated apoptosis of hepatocytes may be increased by HIV-HCV co-infection (Operskalski and Kovacs, 2011).

Following the successful suppression of HIV by HAART in an HIV infected person, the progression rate of fibrosis and necro-inflammatory activity are reduced to levels observed in HCV mono-infected persons. This reinforces the role HIV plays in liver disease progression in HIV-HCV co-infections (Tuyama et al, 2010).

2.6 Prevention and Control of HIV, HBV and HCV Infections

The prevention strategies of HIV, Viral Hepatitis and sexually transmitted diseases are similar. This is because the risk factors for transmission of HIV and Viral hepatitis overlap (CDC, 2009). In the setting of limited public health resources, integrating the prevention services for these viruses is more efficient. In public health settings such as HIV counselling and testing sites, sexually transmitted disease (STD) clinics, substance abuse treatment programs, and prisons; there are many opportunities to prevent HIV and viral hepatitis infections (Buffington and Jones, 2007).

Levels of prevention of diseases can be grouped as primary, secondary and tertiary levels. Primary prevention strategies are used before a person gets a disease. This aims to prevent the disease from occurring. Reduction in prevalence and incidence of a disease can be achieved through primary prevention methods (CDC, 2007). Secondary prevention measures

are resorted to after the disease has occurred but before the person realises that there is something wrong with him/her. Secondary prevention aims at finding and treating diseases early (CDC, 2007). Tertiary prevention is directed towards persons who are already having symptoms of the disease. Tertiary prevention aims at preventing damage and pain that may result from the infection, slowing down the disease progression and preventing complication that may arise from the disease. It also aims at giving better care to persons living with the disease, helping them become healthy again and making them able to do what they used to do (CDC, 2007).

There are two types of exposures to infectious agents. These are occupational and non-occupational exposures (Landovitz and Currier, 2009). The basic way of preventing the transmission of HBV, HCV and HIV is avoiding exposures to the infectious agents. However, hepatitis B immunization and post-exposure prophylaxis are integral components of a complete programme to prevent the infection of HBV, HCV and HIV (CDC, 2001). The medical response given to a person to prevent the transmission of pathogens after potential exposure is known as post-exposure prophylaxis (PEP) (Sharma et al, 2007).

2.6.1 Prevention of mother to child transmission of HIV, HBV and HCV co-infections

Without intervention, the risk of mother to child transmission of HIV is 15%−30% in non-breastfeeding populations. An infected mother increases the risk of transmission to her infant by 5% -20% through breastfeeding. This makes the overall rate of mother to child transmission of HIV 20%−45% in untreated women (WHO, 2010) as compared to <1%-8% in treated women (Rainbow Clinic, 2011). Transmission of HIV from a mother to her child can take place during pregnancy, during labour and delivery or through breastfeeding (Rainbow Clinic, 2011).

Vertical transmission of HCV is generally at 4 to 7% in HIV negative mothers and up to 20% in HIV co-infected mothers (Su, 2005). In women co-infected with HIV and HCV, the exact timing of HCV transmission from a woman to her child is not known (Rainbow Clinic, 2011).

In the absence of immunoprophylaxis, 10%-20% of HBsAg sero-positive women transmit the virus to their newborn children (ACOG, 2007). Vertical transmission is approximately 90% in women who are sero-positive for both HBsAg and HBeAg. Vertical transmission of HBV occurs in up to 10% of neonates when acute infection occurs in the first trimester of pregnancy and in 80%-90% of neonates when the mother gets the acute infection in the third trimester of pregnancy (ACOG, 2007). Available evidence suggests that both in-utero transmission and intrapartum/peripartum transmission can occur. It is generally accepted that mother to child transmission (MTCT) of HBV occur during delivery or near the time of birth. Intra-uterine transmission of HBV occurs in a minority of cases (Jonas, 2009).

A theoretical risk of transmission of HCV through breast milk may exist, but the likelihood seems small. Therefore, unless there is clear nipple trauma/abrasions which may increase the infant's exposure to maternal blood, breastfeeding by HCV positive mothers is acceptable according to current recommendations (Su, 2005).

HIV infected mothers can breastfeed their babies without transmitting the organism to their infants. This can be achieved when they receive anti-retroviral prophylaxis and go through adequate counselling on infant feeding (NACP, 2008). Breastfeeding poses no additional risk for mother to child transmission of HBV in infected hepatitis B carriers after receiving appropriate hepatitis B immune prophylaxis (Wang et al, 2003).

Pregnant women whose HIV and HBV status are unknown should be tested for HIV (NACP, 2008) and HBV (Jonas, 2009). This is because there are possible interventions to prevent mother to child transmission and also to improve the health of the mother (WHO, 2007a).

Routine screening of pregnant women for HCV is not recommended. This is because interventions to prevent vertical transmission are presently not available. Therefore there is no advantage in identifying maternal HCV infection during pregnancy with respect to preventing mother to child transmission. The exception to this appears to be in HIV/HCV co-infected women and therefore HCV testing is recommended for women with HIV positive screening test results (Thorne, 2011).

There are also no advantages in diagnosing HCV during pregnancy in respect to the management of maternal HCV disease (Thorne, 2011). This is because current drug regimen for treating HCV infection is contraindicated in pregnancy due to its effect on developing foetuses (Su, 2005). In addition there may be psychological disadvantages to HCV diagnosis in pregnancy (Thorne, 2011) since there are no measures that can help to reduce the probability of vertical transmission (Thorne, 2011; WHO, 2007a).

High maternal viral load and co-infections are risk factors for mother to child transmission of HIV and HCV. Low CD4 count, the use of invasive devices (such as foetal scalp electrodes) during labour, prolonged rupture of membranes and breastfeeding in the absence of anti-retroviral prophylaxis are additional risk factors for mother to child transmission of HIV (Rainbow Clinic, 2011).

In hepatitis B infected pregnant women, high maternal viral load (Jonas, 2009) and HBeAg positivity are risk factors for mother to child transmission in the absence of hepatitis B immunoprophylaxis (ACOG, 2007).

Delivery by caesarean section for the purpose of reducing vertical transmission of HBV is not presently recommended (ACOG, 2007). There is not enough evidence to support delivery by caesarean section for HCV infected women routinely, for the purpose of preventing mother to child transmission (Rainbow Clinic, 2011). Vaginal delivery is the safest mode of delivery in Ghana for HIV infected women. Caesarean section for HIV infected pregnant women shall be considered on obstetric grounds rather than solely for PMTCT. Where Caesarean section is indicated this must be performed promptly (NACP, 2008).

2.6.1.1 Strategies for prevention of vertical transmission of HIV

The majority of HIV infections in children are acquired from their mothers (WHO, 2010). The global epidemiology of HIV in children reflects that of HIV in women. About 15% of all new HIV infections in Ghana are acquired through mother to child transmission (NACP, 2008). Almost all such infections can be avoided by prevention of mother to child transmission (PMTCT) programmes providing highly effective antiretroviral therapy and antiretroviral prophylaxis interventions (WHO, 2010).

Prevention of mother to child transmission (PMTCT) of HIV in Ghana is defined as a comprehensive family centred continuum of promotive, preventive, clinical and supportive services provided in conjunction with other public health interventions to prevent the transmission of HIV from a mother to her infant (NACP, 2008).

The WHO recommends that the best option for PMTCT is the provision of maternal ARV for life. All HIV-positive pregnant or breastfeeding women should be provided with a course of antiretroviral drugs to prevent mother-to-child transmission. A triple-drug antiretroviral regimen should be taken throughout pregnancy, delivery and breastfeeding. This is continued for life, regardless of CD4 count or clinical stage. Their infants should receive NVP daily or AZT

twice daily from birth (within 6hours to 12hours) or as soon as possible until four (4) weeks to six (6) weeks of age (WHO, 2013b).

2.6.1.2 Prevention of mother to child transmission of HBV in HIV infected women

Hepatitis B screening should be part of routine prenatal testing of all HIV infected women. HIV/HBV co-infected women should have 3TC as part of the ARV combination administered to them because it is effective against both viruses. HBV vaccination provides up to 95% protective efficacy for children born to chronically infected mothers (WHO, 2007a).

Infants born to HBsAg positive mothers should receive 0.5ml of hepatitis B imunoglobulin along with single antigen HBV vaccine within twelve (12) hours of birth. The normal HBV vaccination schedule (at 0, 1 and 6 months) should then be continued for such children (CDC, 2005; WHO, 2007a). The initial vaccination following delivery is associated with lower immunogenicity in children weighing less than 2kg. For such children, HBV vaccination should be given in four doses (at birth, one (1) month, two (2) months to three (3) months and six (6) to seven (7) months) (WHO, 2007a). There should be HBsAg and HBsAb testing at nine (9) and eighteen (18) months for children born to HBsAg positive mothers. The entire three vaccine series should be repeated if the HBsAb level is below 10mIU/ml. At delivery if the HBsAg status of a woman is unknown but is realized to be positive, Hepatitis B immune globulin (HBIg) can be administered up to seven (7) days after birth (CDC, 2005; WHO, 2007a).

HBV vaccine and immunoglobulin should be administered to infants weighing less than 2kg who are born to women of unknown HBsAg status. Children born to HBsAg-negative

mothers should receive their first vaccination at the hospital; however this should be delayed until one (1) month of age for children who weigh less than 2kg (WHO, 2007a).

2.6.1.3 Prevention of mother to child transmission of HCV in HIV infected pregnant women

There are no interventions currently to prevent mother to child transmission of HCV even though several risk factors for vertical transmission have been identified. HCV treatment is considered before pregnancy for women of child bearing age whenever possible (Su, 2005). Steps such as a pregnancy test before treatment and counseling of partners to avoid pregnancy during treatment and until at least six months after treatment will help prevent ribavirin embryopathy (WHO, 2007a).

2.7 Laboratory Diagnosis of HIV, HBV and HCV Infections

Direct detection methods and serological diagnostic methods are available for the laboratory diagnosis of HIV infection (Simmonds and Peutherer, 2002b), HBV infction (Simmonds and Peutherer, 2002a) and HCV infection (Morgan-Capner and Simmonds, 2002). Blood is the specimen of choice for the diagnosis of HBV (Krajden et al, 2005), HIV (Fearon, 2005) and HCV infection (Morgan-Capner and Simmonds, 2002).

2.7.1 Serological diagnostic methods

Serological tests for viral antigens and antibodies are generally used for diagnostic screening and can be performed on either serum or plasma (Krajden et al, 2005). Antibody testing is

technically simple. This has favoured its use for general screening and diagnostic testing (Morgan-Capner and Simmonds, 2002). Viral antigens and specific antibodies in body fluids are detected based on the use of sandwich enzyme immonoassays. Circulating antibodies or antigens are captured onto the wells of microtitre plates, microbeads or specific holders adapted to close automated devices by the use of recombinant antigens or antibodies. The detection of the presence of antibodies or antigens in the test sample is based on the use of antibodies (in the case of antigen) or anti-antibodies (in the case of antibody) labelled with an enzyme that catalyzes the transformation of a substrate into a coloured compound. The amount of antigen or antibody in the plasma is proportional to the optical density (OD) ratio (sample OD/internal control OD) (Chevaliez and Pawlotsky, 2008).

2.7.1.1 Serological diagnosis of HCV infection

A number of immunoassays have been developed to detect anti-HCV immunoglobulin G (IgG) in serum or plasma. Anti-HCV antibodies appear on average two (2) to eight (8) weeks after the acute phase of infection. It persists for life in patients who develop chronic HCV infection (Elliott et al, 2006)

The detection of anti-HCV antibodies does not help to distinguish between acute HCV infection, chronic HCV infection (Beltrami et al, 2000), acute exacerbation of chronic HCV in persons with chronic hepatitis C and acute hepatitis of other origins in a patient with chronic HCV infection (Chevaliez and Pawlotsky, 2008). Anti-HCV IgM has been reported in 50% to 70% of patients with chronic hepatitis C (Hsu and Greengberg, 1994). Therefore it cannot be used as a reliable marker for the diagnosis of acute HCV infection.

However, the estimation of the avidity index of anti-HCV IgG within eight (8) days following the onset of clinical symptoms may be useful in identifying actual acute HCV infection cases (Chevaliez and Pawlotsky, 2008). Acute HCV infection may be diagnosed by measuring anti-HCV IgM titre at least three times from the fifth to the fifteenth day from the onset of the symptoms (Sagnelli et al, 2005). Competitive enzyme-linked immunosorbent assay using genotype-specific antigens can be used to determine HCV genotypes (1 to 6) serologically (Pawlotsky et al, 1997). However this method cannot be used to determine the HCV subtype (Chevaliez and Pawlotsky, 2008).

2.7.1.2 Serological diagnosis of HIV

Highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) tests are currently available. This has made it possible for seroconversion to be detected within two to three weeks of infection in the majority of cases. HIV- specific antibodies can be detected in the plasma or serum of infected persons (Fearon, 2005).

HIV infection can be diagnosed based on the detection of antibodies to the virus (anti-HIV) because HIV is invariably persistent. An HIV-infected person will remain infected; therefore the detection of anti-HIV in the serum or plasma of a patient indicates HIV infection. HIV antigens can be obtained from cloned recombinant HIV gag, pol and env genes expressed in *Escherichia coli* or synthetic peptides. These antigens are used in current available HIV serological tests (Simmonds and Peutherer, 2002b). All positive anti-HIV screening tests should be confirmed with another assay (Fearon, 2005) which uses different viral antigens (Simmonds and Peutherer, 2002b). The most common confirmatory HIV serological test is the western blot (Beltrami et al, 2000; Fearon, 2005).

2.7.1.3 Serological diagnosis of HBV

Very efficient enzyme immunoassays for the detection of HBV markers in the serum or plasma are available (Chevaliez and Pawlotsky, 2008). Serological markers of HBV include the hepatitis B surface antigen (HBsAg) (Beltrami et al, 2000) which is present in excess in the plasma of infected persons (Krajden et al, 2005). The standard screening test for the detection of HBV infection is for HBsAg. HBsAg is detected several weeks before the onset of clinical and biological symptoms (Beltrami et al, 2000; Simmonds and Peutherer, 2002a). The HBsAg titre is highest at the height of liver damage (Simmonds and Peutherer, 2002a).

HBsAg can be detected early during acute infection, on average 6 to 10 weeks after exposure. Improvement in the analytical sensitivity and specificity of commercially available enzyme immunoassays has made it possible to detect at least 0.15 nanograms (ng) per milliliter (mL) of circulating HBsAg (Ly et al, 2006). This has reduced the window period of HBV infection by up to nine (9) days. Currently, available HBsAg detection assays have a specificity of more than 99.5%. However, in pregnant women, autoimmune disease and chronic liver diseases of other causes, false positive results may occur. False positive results can also sometimes be observed in heparinized, hemolyzed (hemoglobin above 1.4 g/dL) or icteric (bilirubin above 52.8 μmol) blood specimens. In order to eliminate false-positive results, it is recommended that the initial HBsAg results should be confirmed by neutralization (Chevaliez and Pawlotsky, 2008).

Chronic HBV infection is defined as persistence HBsAg positive results for more than six months after exposure (Simmonds and Peutherer, 2002a). However, HBsAg may not be detectable during chronic infection in certain instances such as;

- in low-replication asymptomatic HBV carriers

- in the case of HBV variants bearing nucleotide substitutions in the S gene leading to the synthesis of an HBsAg that is not recognized by commercial assays
- when infection resolves spontaneously or after successful antiviral therapy in chronic HBV-infected patients who may subsequently achieve an HBs seroconversion;
- in hepatitis delta HBV-co-infected patients, where hepatitis delta virus most often inhibits HBV replication and expression (Chevaliez and Pawlotsky, 2008).

HBsAg can be quantified using fully automated chemiluminescent microparticle immunoassay. This comes with improved precision, reliability, technical simplicity, rapid turnaround time and high-speed throughput (Cabezas-Fernandez and Cabeza-Barrera, 2012). It may also provide a means to establish the prognosis of antiviral therapy in the future (Chevaliez and Pawlotsky, 2008).

Anti-hepatitis B surface (anti-HBs) antibodies and anti-hepatitis B core (anti-HBc) antibodies are also serological markers of HBV infection (Krajden et al, 2005). The presence of anti-HBs when anti-HBc is also present marks the recovery from chronic HBV infection. Such a person has a life-long immunity against HBV. The presence of Anti-HBs alone marks immunity developed as a result of vaccination (CDC, 2012). Anti-HBs antibodies may become undetectable several years after the acute episode because its titre often varies over time (Chevaliez and Pawlotsky, 2008).

Anti-HBc antibodies are detected early during HBV infection (Krajden et al, 2005). There are two isotypes of anti-HBc antibodies (Simmonds and Peutherer, 2002a). Anti-HBc immunoglobulin G, rises parallel to HBsAg at the acute stage and persist for life-long irrespective of the outcome of the infection. Anti-HBc immunoglobulin M is a marker of acute HBV infection. However, during the immune elimination phase of chronic hepatitis B and during exacerbations in inactive carriers, low levels of anti-HBc IgM can be detected.

Unlike anti-HBs antibodies, anti-HBc IgG are not neutralizing in vivo. False negative anti-HBc antibodies result may occur rarely in immunocompromised patients (Chevaliez and Pawlotsky, 2008).

HBV-2 is a rare variant of the hepatitis B virus which has a defect in the core gene.This makes the HBV-2 variant unable to elicit detectable immune response to HBc antigen in immunocompetent persons (Chevaliez and Pawlotsky, 2008).

It is possible for anti-HBc antibodies to be the only detectable HBV marker in an individual. This situation may occur:

- In low-replication asymptomatic chronic HBV carriers with low HBsAg production. This is common in areas of low HBV prevalence and in HBV co-infection with HIV or HCV.
- In individuals with immunity after recovery from prior infection. In such persons, anti-HBs have reduced to undetectable levels but anamnestic response can be observed after one dose of HBV
- In some persons especially those from areas of low HBV prevalence, false positive anti-HCV results may be obtained
- In persons at the window phase of acute HCV infection, anti-HBc (IgM) may be the only detectable serological marker vaccine (Lok and McMahon, 2007).

Hepatitis B e antigen (HBeAg) and anti-HBe antibodies are another set of serological markers of HBV (Cabezas-Fernandez and Cabeza-Barrera, 2012). The preC/C gene of the HBV codes for the HBe protein that bears the HBeAg. This protein is secreted by infected cells under various soluble forms that vary from 16kDa to 20kDa in size. The presence of HBe protein is associated with immune tolerance, high-level HBV replication and high infectivity, although it does not appear to be essential for the lifecycle of HBV. The HBeAg

can be detected six (6) to twelve (12) weeks after exposure during acute HBV infection (Chevaliez and Pawlotsky, 2008).

In acute HBV infection, the clearance of HBeAg is associated with a decrease of viremia and an aminotransferase flare. This is followed by the appearance of anti-HBe antibodies. Persistence of HBeAg is usually observed in patients who develop chronic infection (Chevaliez and Pawlotsky, 2008).

Two types of chronic HBV infection can be observed taking into consideration the presence of HBeAg. This includes, HBeAg –negative and HBeAg-positive chronic hepatitis B. In HBeAg-positive chronic hepatitis B, the presence of HBeAg in peripheral blood is normally associated with a high HBV deoxyribonucleic acid (DNA) level and no anti-HBe antibodies are found in the plasma or serum of the infected individual (Liang, 2009). In this case, the preC/C gene has a wild-type sequence (Chevaliez and Pawlotsky, 2008).

In HBeAg-negative chronic hepatitis B infected individuals, the virus has nucleotide substitutions in the precore region and/or in the basal core promoter region of the preC/C gene. The mutation may lead to the insertion of a stop codon in the precore sequence which prevents the synthesis of HBe protein. It can also lead to the down regulation of HBe protein production of up to 70%. In the same patient, different variants bearing precore and/or basal core promoter mutations can be detected in variable proportions. HBeAg-negative variants are usually detected during the immune clearance of chronic HBV infection (Chevaliez and Pawlotsky, 2008). There is seroconversion from HBeAg to anti-HBe antibodies during the immune elimination phase. The outcome of the serconversion phase is either HBeAg negative chronic hepatitis B or inactive carrier state (Liang, 2009).

In the inactive carrier state, the aminotransferase levels are normal and the HBV DNA level is low (<2000 IU/mL) or undetectable (Puoti, 2013).

2.7.2 Direct detection methods

Direct detection methods used in the diagnosis of viruses are based on the detection of genetic material of the virus (molecular methods) and a number of non molecular methods such as quantitative viral antigen detection (Berger and Preiser, 2002; Morgan-Capner and Simmonds, 2002) and viral culture (Berger and Preiser, 2002; Simmonds and Peutherer, 2002).

Whereas non-molecular methods are very useful in certain context, they may be either too cumbersome or impossible to perform, or of inadequate sensitivity to be clinically useful (Berger and Preiser, 2002).

There are two categories of molecular biology techniques that can be used for viral genome identification and quantification. This includes target amplification such as polymerase chain reaction (PCR) and signal amplification such as hybrid capture or the branched DNA assay (Chevaliez and Pawlotsky, 2008).

In clinical practice, detecting and quantifying viral genetic material (viral load) is useful to:

- Diagnose viral infection
- Assess the patient's prognosis
- Estimate the patient's infectivity (the risk of transmission) (Berger and Preiser, 2002).
- Identify patients who need to be put on antiviral therapy and monitor the therapy (Chevaliez and Pawlotsky, 2008; Cabezas-Fernandez and Cabeza-Barrera, 2012)
- Assess the potential for emergence of viral resistance (Chevaliez and Pawlotsky, 2008; Luft et al, 2011).

2.7.2.1 Direct detection of HBV

Apart from the detection and quantification of HBV-DNA, there are three other molecular assays used in the diagnosis and management of HBV infection. These are genotyping assays, drug resistance tests and core promoter/precore mutation assays (Cabezas-Fernandez and Cabeza-Barrera, 2012).

HBV-DNA can be detected within few days after infection (Whalley, 2001). The presence of HBV-DNA in serum or plasma is a reliable marker of HBV replication (Chevaliez and Pawlotsky, 2008). It indicates active HBV infection. The presence of HBV-DNA correlates with the infectivity of the patient depending on the viral load (Krajden et al, 2005).

2.7.2.2 Direct detection of HCV

There is a significant window period between exposure to HCV and development of antibodies which is detectable by the best available ELISA methods (Morgan-Capner and Simmonds, 2002).

HCV RNA can be detected in peripheral blood as early as one (1) week after infection (Kamili et al, 2012). This is about one (1) month before the appearance of anti-HCV antibodies (Chevaliez and Pawlotsky, 2008). This has necessitated the use of direct detection methods for detecting HCV antigens or RNA sequences. These methods are more effective in diagnosing HCV infection in acute hepatitis and in immunosuppressed individuals who do not put up detectable antibody response (Morgan-Capner and Simmonds, 2002).

The detection of HCV RNA in plasma or serum is the most reliable marker of diagnosing active HCV infections (Kamili et al, 2012).

PCR is the most commonly used direct detection method for diagnosing HCV infection (Morgan-Capner and Simmonds, 2002). However, HCV viral protein can be detected using ELISA-based methods. This serves as an alternative to the nucleic acid detection. An example is the ortho trak-C assay for the detection of HCV core protein (Daniel et al, 2007).

2.7.2.3 Direct detection of HIV

HIV RNA and DNA can both be detected by PCR (Pasternak et al, 2008). The HIV RNA sequences are found in extracellular particles in the plasma of infected persons. However, the HIV proviral DNA can be detected in the mononuclear cells found in the peripheral blood of infected individuals (Simmonds and Peutherer, 2002b).

The HIV DNA-PCR allows for the detection of viral DNA integrated into the host cell genomic DNA.This method is particularly useful in diagnosing HIV in infants born to HIV infected mothers. Infants born to HIV-infected mothers may carry maternal antibody for up to fifteen (15) months of age. HIV-DNA PCR is also useful in testing patients who have aggammaglobulinemia and in patients who have symptoms of advanced HIV infection but do not have detectable HIV-specific antibodies (Fearon, 2005).

HIV RNA levels denote the degree of virus replication in the patient (Simmonds and Peutherer, 2002b). Quantitative HIV PCR (RNA) testing (viral load) is essential in monitoring the disease progression of HIV (Luft et al, 2011).

HIV genotyping is a new way of tracking the development of drug resistance. It also provides guidance in modification of antiretroviral drug selection (Fearon, 2005).

HIV has the p24 antigen as part of its virion core. This can be present in peripheral blood when there is active viral replication. The p24 antigenaemia may not be detected in all cases since it is of short duration. The antigen test becomes negative as the antibody response builds up. It may however reappear in late infection (Simmonds and Peutherer, 2002b).

There are enzyme immunoassays for the detection of the p24 antigen in the serum (Hashida et al, 2000). Antibodies are used to capture the disrupted p24 antigen from patient serum. When a patient test positive to the p24 antigen but test negative to the HIV antibody screen; a follow up antibody test should be requested. This will be positive within few weeks after the initial antibody screen in patients undergoing seroconversion (Fearon, 2005).

2.8 Treatment of HIV, HBV and HCV Infections

2.8.1 Treatment of HIV infection

Antiretroviral drugs are used in the treatment of HIV. These are grouped into six major types based on how they interfere with the replication of the HIV (Thacker, 2011). These groups include:

- Entry inhibitors which acts by impeding the ability of the virus to bind with receptors or co-receptors on the outer surface of the cell. When the virus is prevented from binding to the receptors, it cannot infect the cell
- Fusion Inhibitors impedes the capability of the virus to fuse with the host cell membrane. This prevents HIV from entering a cell (Lobritz et al, 2010)
- Reverse Transcriptase Inhibitors which prevents the conversion of single-stranded HIV RNA into double-stranded HIV DNA by the HIV enzyme called reverse transcriptase. This process is known as reverse transcription. There are two types of

reverse transcriptase inhibitors. The nucleoside reverse transcriptase inhibitors (NRTIs) are faulty DNA building blocks. These stop HIV DNA synthesis when added to a growing HIV DNA chain. This is because after a copy of the faulty DNA is added no correct DNA building block can further be added on. The non-nucleoside reverse transcriptase (NNRTIs) acts by binding to the enzyme reverse transcriptase and therefore interfering with its ability to convert HIV RNA into HIV DNA (NIAID, 2009)

- Integrase Inhibitors which prevents the incorporation on the viral genetic material into the DNA of the infected cell by blocking the HIV enzyme called integrase.
- Protease inhibitors (PIs) which acts to prevent new viral particles from being assembled. This is achieved by interfering with the activity of the HIV enzyme called protease. The HIV protease cleaves long chains of HIV proteins into smaller individual proteins thereby making assembling of new HIV viral particles possible (Bean, 2005) and
- Multi-class Combination Products which are combinations of ARVs from two or more classes into a single product (NIAID, 2009).

HIV can develop resistance to a particular class of antiretroviral drugs. This can be prevented by administering to HIV infected persons a combination of ARVs from at least two different classes. This approach is called highly active antiretroviral therapy (HAART) (NIAID, 2009).

The early initiation of ART is of immense benefit both clinically and in HIV prevention. It is associated with improved survival and in reducing the incidence of HIV infection in the community (CDC, 2013). The WHO recommends that ART should be started immediately regardless of CD4 count in the following situations;

- In individuals who are HIV/HBV co-infected with severe chronic liver disease
- In individuals who are co-infected with HIV and TB
- In serodiscordant couples the uninfected partner should be offered ART to reduce the risk of getting infected
- In HIV infected pregnant or breastfeeding women and
- In children under five (5) years of age (WHO, 2013b).

In the general adult HIV infected population, ART is started when the CD4 count is ≤ 500cells/µl. However, priority is given to individuals who are in WHO clinical stage three (3) or four (4) and individuals whose CD4 count is ≤ 350cells/µl (WHO, 2013b).

HIV positive patients who are put on ART should be monitored for clinical, immunological and virological treatment failure. HIV viral load and CD4 count are used to monitor the virological and immunological treatment failure respectively. Second line ART drug regimen is resorted to when there is treatment failure during therapy with the first line ARVs (Ingole et al, 2013).

2.8.2 Treatment of HBV infection

There are eight medicines that are currently available for the treatment of hepatitis B. These include interferon-α, pegylated-interferon, lamivudine (3TC), entecavir, Tenofovir (TDF) and emtricitabine (FTC) which have activity against both HIV and HBV. Adefovir and telbivudine are also used in the treatment of HBV but have no activity against HIV.

Entecavir, interferon-α and pegylated-interferon are contraindicated in pregnant and breastfeeding women (Rockstroh et al, 2008).

The desired goal for the treatment of hepatitis B infection is to achieve the clearance of HBsAg with the appearance of HBsAb (seroconversion). This result is however not achieved in the majority of cases. Long-term suppression of HBV replication to decrease liver inflammation and prevent or delay progression of hepatic fibrosis is more achievable (Rockstroh et al, 2008).

2.8.3 Treatment of HCV infection

There is the possibility of eliminating HCV within a defined treatment period. Therefore HCV treatment should be considered for each patient when the benefit of the therapy is more than the possible risks involved (Rockstroh et al, 2008). The treatment of choice for HCV infection is a combination of ribavirin and pegylated interferon-α. These medicines are administered according to the body weight of the patient (Strader et al 2004; Rockstroh et al, 2008).

The urgency for the treatment of HCV in persons co-infected with HIV is greater than in persons who are HCV mono-infected. HIV/HCV co-infection is associated with lower SVR. HCV infection is associated with increased risk of hapatotoxicity from HAART. Treatment of HCV in HIV/HCV co-infected persons might help improve the tolerability of HAART. The dosage for management of HCV in HIV/HCV infected persons may differ from that used in managing the HCV mono-infected person.

However, there are not enough data to base recommendations on. Therefore, the dosage used in managing the HCV mono-infected persons is used for the HIV/HCV co-infected persons (Strader et al 2004).

The main aim of HCV treatment is to achieve sustained virological response (SVR). Sustained virological response (SVR) means that HCV RNA is undetectable 24 weeks after HCV treatment. The HCV RNA levels should be evaluated using ultrasensitive PCR assays (Rockstroh et al, 2008).

In HIV/HCV co-infected patients who have CD4 count less than 200cells/µl (severe immunodeficiency), the CD4 count should be improved by the use of HAART before treatment for chronic HCV infection can be started. However, when chronic HCV infection is detected early in the course of HIV (before the initiation of HAART), treatment for HCV is advised (Rockstroh et al, 2008).

Treatment for HCV is recommended when two HCV RNA tests carried out one week apart and within twelve weeks after the initial exposure in patients with acute HCV infection give positive results.

Treatment of acute HCV infection gives better SVR rates than the treatment of chronic HCV infection. Didanosine, stavudine and zidovudine should be avoided during the treatment of HCV using pegylated interferon and ribavirin (Rockstroh et al, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

Techiman was chosen as one of the study sites because it is a town with a relatively high commercial activity. Hwidiem was also chosen because it is a mining area. Like all similar towns, mining towns are associated with behaviours such as commercial sex activities, multiple sexual partners, drug abuse and other practices that may expose a person to HIV, HBV and HCV infections. There is a well-resourced district hospital in each of these towns.

3.2 Study Design

This was a hospital based case -control study.

3.3 Subjects

Informed consent was sought from pregnant women who tested positive to HIV at the antenatal clinic and HIV-infected pregnant women who visited the ART clinic at the St. Elizabeth Hospital, Hwidiem and the Holy Family Hospital Techiman in the Brong-Ahafo Region of Ghana. Those who consented were enrolled into the study as study participants. A control group consisting of HIV negative pregnant women were also enrolled into the study.

3.3.1 Inclusion criteria

Two groups of participants (a study group and a control group) were enrolled into this study. Only pregnant women were included in this study.

- For the study group, HIV-infected pregnant women will be included in the study
- For the control group, HIV-negative pregnant women

3.1.2 Exclusion criteria

All non-pregnant women were excluded from this study

- For the Study population, HIV-negative pregnant women will be excluded
- For the control population, HIV-infected pregnant women will be excluded

3.4 Sample Size

The sample size was calculated using the Cochran's sample size formula (Cochran, 1977)

$n = \frac{Z^2(Pq)}{d^2}$

Z = Reliability Co-efficient with 95% CI

P= Proportion variance available from previous data where q= 1-p

d= the desired or required size of standard error allowed.

Z – Value of 95% confidence interval is 1.96 (Z=1.96)

The prevalence of HIV among pregnant women in the Brong-Ahafo Region is 2.6% (NACP, 2011)

P=2.6 or 0.026

q=1-p

=1-0.026

=0.974

d=0.05 or 5%

Z=1.96

$$n = \frac{(1.96)2\ (0.026\text{x}0.974)}{(0.05)^2}$$

$$n = \frac{(3.8416)(0.0253)}{0.0025}$$

$$n = \frac{0.0972}{0.0025}$$

=38.87

≈39

The statistical calculation for the number of participants to make this research work meaningful is 39 HIV-infected pregnant women. However a total of 124 participants were enrolled into the study. This included a study group of 74 pregnant women who were identified as HIV-infected before or during pregnancy and 50 HIV-negative pregnant women.

3.5 Sampling and Research Techniques

The demographic information as well as information on possible risk factors for HIV/HBV and HIV/HCV co-infections was collected from each participant using a standardized questionnaire (see appendix). Venous blood sample (5mls) was taken from each participant under strict aseptic conditions. Hepatitis B serological markers and antibodies to the hepatitis C virus were determined, using test kits that uses the ELISA technique. The baseline CD4 count of the study group was determined using BD Facs Count Analyzer (USA). The full blood count of participants was run using the Sysmex KX N21 analyzer (Japan).

In the third trimester of pregnancy, at delivery or soon after delivery, 5mls of blood specimen was taken from the study group and HIV RNA viral load was determined. Information on ART was obtained from the patient's folder.

3.6 Ethical approval

The Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) and Komfo Anokye Teaching Hospital, Kumasi, Ghana approved the protocol for the study.

3.7 Research Instruments and Assays

3.7.1 Hepatitis B profile

Hepatitis B Profile Test kit, from Guangzhou Wondfo Biotech Co. Ltd, Luogang District, China was employed.

The cassette test for the detection of hepatitis B surface antigen (HBsAg) takes advantage of the formation of a visible spot by precipitating immunocomplexes.

HBsAg and HBeAg are estimated with a dual-antibody sandwich enzyme linked immuno sorbent assay (ELISA) method. HBsAb is estimated with a dual-antigen sandwich method whiles HBeAb and HBcAb are estimated with a neutralization competitive inhibition ELISA method.

3.7.2 Testing for Anti-HCV

Anti-HCV Test-Kit, from Guangzhou Wondfo Biotech Co. Ltd, Luogang District, China was utilized.

The one step wondfo test for the detection of anti-HCV takes advantage of the formation of a detectable spot by precipitating immunocomplexes. This test is based on double antigen sandwich ELISA method.

The specimen is absorbed into the test device by capillary action when the device is immersed into it. The absorbed specimen mixes with an antigen-dye conjugate and flows across a pre-coated membrane. The HCV antibodies in the specimen bind to the antigen-dye conjugate and are captured by antigen immobilized in the test region (T) of the device. This produces a coloured test band and indicates a positive test results.

When there are no HCV antibodies in the plasma or its level is below the target cut off, no visible coloured line is developed in the test region.

To serve as control to the procedure, a coloured line develops at the control (C) region if the test is performed correctly.

3.7.3 Full Blood Count

Sysmex KX N21 Haematology Analyzer, from Symex Corporation, Japan was used.

Blood cells are measured based on direct detection method. Aspirated blood sample is measured, diluted and fed into a transducer. The transducer has an aperture which is positioned between two electrodes. Direct current flows between these electrodes. As the suspended blood cells pass through the aperture, it causes direct current resistance to change between the electrodes. The blood cell size is detected as electrical pulses, as the direct current resistance changes. A non-cyanide haemoglobin analysis method is used to measure the haemoglobin. Haemoglobin is rapidly converted to oxyhemoglobin and measured.

3.7.4 CD4 Count

BD FACSCount Systems (USA) was used.

Flourochrome-labeled antibodies in the reagent bind specifically to lymphocyte surface, when whole blood is added to the BD FACSCount CD4/CD3 reagent. After incubation, a fixative solution is added to the reagent tubes. The sample is run on the analyzer. During the run process, the cells come into contact with laser light which causes the flourochrome-labeled cells to fluoresce. The fluorescent light provides the information for the instrument to count the cell.

3.7.5 Human immunodeficiency virus type-1 (HIV-I) viral load

COBAS® AmpliPrep/COBAS® TaqMan Analyzer, from Roche Molecular System, USA was used.

The HIV-1 Test is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. Specimen preparation is automated using the COBAS AmpliPrep Instrument whereas the amplification and detection is automated using the COBAS TaqMan Analyzer or the COBAS TaqMan 48 Analyzer. The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target.

3.5 Data Analysis

The statistical analysis was done using Stata/I/C 12.0. Univariable and multivariate logistic regression analysis were used to obtain unadjusted and adjusted odds ratio respectively. Also univariate and multivariate regression analysis were used to obtain unadjusted and adjusted regression coefficient. Continues variables between two groups was compared with t-test and presented as mean ± SD (standard deviation). Categorical variable was presented as frequency (n) and percentages (%). A p-value <0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 General Characteristics

Out of a total of 124 participants, 74(60%) were HIV infected whereas 50(40%) were HIV negative. The mean age of the participants was 28.19 ± 6.32 years. The HIV-infected participants (the study group) had a mean age of 29.18 ± 6.10 years whereas the mean age of the HIV-negative participants (the control group) was 26.98 ± 6.42 years. The control group were younger than the study group. The study group had a higher body mass index (BMI) and platelet count as compared to the control group. They however had a lower haemoglobin (Hb) level and white blood cell (WBC) count as compared to the control group (Table 1).

Table 1: The mean values of the age, BMI, Hb, WBC and platelet count of the study participants

Parameters	HIV-Infected	HIV-Negative	Total population
Age (Years)	29.18 ± 6.10	26.98 ± 6.42	28.19 ± 6.32
BMI	24.90 ± 3.91	23.17 ± 3.55	24.07 ± 3.82
Hb	9.7 ± 1.81	10.75 ± 1.68	10.16 ± 1.82
WBC	5.60±1.81	7.23 ± 2.13	6.31 ± 2.11
Platelet count	220.62 ± 86.73	209.38 ± 65.59	215.69 ± 78.03

The values are presented as X ± SD, where X= mean and SD= standard deviation.

The data for some of the parameters could not be generated for some participants. Throughout this chapter, all the analysis were done taking into consideration only participants with data available for a particular parameter. The various parameters have been stratified according to the HIV status of the participant (Table 2).

Table 2: The general characteristics of the participants according to their HIV status

Parameters	HIV-Infected N=74 n (%)	HIV-Negative N=50 n (%)
Age group		
15-19 yrs	3 (4.9)	8 (16)
20-30 yrs	37 (60.7)	26 (52)
>30	21 (34.4)	16 (32)
History of sharing sharps		
Ever	26 (39.4)	8 (16)
Never	40 (60.6)	42 (84)
History of blood transfusion		
Ever	10 (14.9)	4 (8)
Never	57 (85.1)	46 (92)
BMI		
Underweight	2 (3.7)	3 (6)
Normal	26 (48.15)	38 (76)
Overweight	26 (48.15)	9 (18)
Timing of HIV diagnosis		
Before pregnancy	17 (25.4)	
During pregnancy	57 (74.6)	N/A
If during pregnancy at what stage		
First trimester	7 (12.3)	N/A
Second trimester	34 (59.7)	
Third trimester	16 (28.1)	
Marital status		
Single	17 (26.6)	9 (18)
Married	47 (73.4)	41(82)
Occupation		
Formally employed	2 (3.3)	3 (7.5)
Informally employed	46 (75.4)	36 (90)
Unemployed	13 (21.3)	1 (2.5)

Records not available for age group (14), history of sharing sharps (9), history of blood transfusion (8), BMI (23), timing of HIV diagnosis (8), marital status (11), occupation (24), blood group (12). N= the total number of participants in a particular group whereas n= the number of participants who fall into particular parameter. N/A= not applicable.

4.2 Prevalence of HBV and/or HCV Co-Infection with HIV

4.2.1 Prevalence of HIV/HBV co- infection

The prevalence of hepatitis B defined by the presence of HBsAg was 14.9% among the HIV-infected participants and 10% among the HIV negative participants. The prevalence of HBeAg was also higher in the HIV infected group as compared to the control group. However, the prevalence of HBsAb, HBeAb and HBcAb was higher in the control group as compared to the HIV-infected group. From the univariate logistic regression analysis, HIV-infected participants were significantly less likely to sero-convert from HBeAg to HBeAb. All the other HBV serological markers did not have statistically significant association with the HIV sero-status of participants (Table 3).

Table 3: The prevalence of HBV serological markers stratified by the HIV status

Serological Marker	HIV-Infected Participants N=74 n (%)	HIV-Negative Participants N=50 n (%)	OR(95% CI)	P-Value
HBsAg	11 (14.9)	5 (10)	1.60 (0.51- 4.84)	0.431
HBsAb (Anti-HBs)	2 (2.7)	2(4)	0.67 (0.091- 4.90)	0.690
HBeAg	2 (2.7)	0 (0)	*0.03 (0.02- 0.07)	0.245
HBeAb (Anti-HBe)	6 (8.1)	15(30)	0.21 (0.07- 0.58)	0.003
HBcAb (Anti-HBc)	22 (29.7)	16 (32)	0.90 (0.41-1.95)	0.788

* = Regression Coefficient

4.2.2 Prevalence of HIV/HCV Co-Infection

The prevalence of anti-HCV was lower in the HIV-infected participants as compared to that observed in the HIV-negative participants (Table 4).

Table 4: Anti-HCV prevalence in HIV-infected and HIV-negative participants

HCV serological marker	HIV-Infected participants N=74 n (%)	HIV-Negative participants N=50 n (%)	OR (95% CI)	P-Value
Anti-HCV	3 (4.1)	6 (12)	0.31 (0.07- 1.30)	0.110

4.2.3 Prevalence of HIV/HBV/HCV Triple Infection

None of the participants in this study tested positive to both HBsAg and anti-HCV in the HIV-infected population.

4.4 Possible Risk Factors for HIV/HBV or HIV/HCV Co-infection

A univariate logistic regression analysis of risks factors such as the age, history of sharing sharp objects, history of blood transfusion, marital status and occupation showed that none of these factors significantly increased the risk of getting HIV/HBV or HIV/HCV co-infection among the participants (Table 5).

Table 5: Logistic regression analysis of risk factors for HIV/HBV and HIV/HCV co-infections

Possible Risk Factors	HIV/HBV Co-Infection [N=11]			HIV-HCV Co-infection [N=3]		
	n (%)	**OR (95% CI)**	**P**	**n (%)**	**OR (95% CI)**	**P**
Age group (years)						
15-19	1 (12.5)	1		0 (0)	1	
20-30	6 (75)	0.19 (0.11, 3.54)	0.268	1 (33.3)	0.26 (0.02, 3.1)	0.289
>30	1 (12.5)	0.05 (0.02, 1.53)	0.086	2 (66.7)	1	
History of sharing sharps						
Never	4 (40)	1		1 (33.3)	1	
Ever	6 (60)	2.84 (0.71, 11.31)	0.138	2 (66.7)	3.39 (0.29, 39.50)	0.330
History of blood transfusion						
Ever	7 (70)	3 (0.63, 14.38)	0.169	1 (33.3)	3 (0.25, 36.62)	0.389
Never	3 (30)	1		2 (66.7)	1	
Marital status						
Single	3 (37.5)	1		2 (66.7)	1	
Married	5 (62.5)	0.5 (0.1, 2.46)	0.406	1 (33.3)	0.15 (0.01, 1.81)	0.136
Occupation						
Unemployd	3 (3.3)	1		1 (50)	1	
Employed	6 (66.7)	0.49 (0.1, 2.30)	0.364	1 (50)	0.26 (0.02,4.48)	0.354

HIV/HBV co-infection records not available for age (3), history of sharing sharps (1), history of blood transfusion (1), marital status (3) an occupation (2). HIV/HCV co-infection record not available for occupation (1). OR= Odds Ratio. N= the total number of participants in a particular group whereas n= the number of participants who fall into particular parameter

4.5 Immunological Properties, Virological Properties and the use of ARVS among Participants

Out of the 74 HIV-infected pregnant women, 56 (75.7%) had records on the anti-retroviral therapy. One (1) person (1.79%) received dual therapy (3TC + AZT), whereas 98.2% received a triple drug therapy (3TC+AZT+EFV (3.57%), 3TC+AZT+NVP (82.14%), 3TC + TDF + NVP (1.79%).

The assessment of the baseline CD4 count showed that the majority of the HIV/HBV co-infected participants had a baseline CD4 count ≤ 350 cells/µl whereas, the majority of the HIV/HCV co-infected and the HIV mono-infected participants had CD4 count > 500 cells/µl.

Table 6: CD4 groupings according to the co-infection status participants

CD4 Count (Cells/µl)	**HIV/HBV Co-Infected N=11**	**HIV/HCV Co-Infected N=3**	**HIV Mono-Infected N=60**
	n (%)	n (%)	n (%)
≤ 350	5 (55.6)	0	11 (25.0)
350-500	1(11.1)	1(33.3)	13 (29.5)
>500	3 (33.3)	2 (66.7)	20 (45.5)

Records not available for HIV/HBV co-infection (2), HIV mono-infected (16)

The mean CD4 count of the HIV/HBV co-infected participants was the lowest, whereas that of the HIV/HCV co-infected participants and the HIV-mono-infected participants was similar. The mean CD4 count of the HBeAg positive participants was 185± 10 (Table 4.5b).

Table 7: The mean viral load and mean CD4 count stratified by HIV/HBV and HIV/ HCV co-infection status

Category of participants/ Parameter	**MeanCD4 Count (cells/µl)**	**Mean Viral Load (copies/ml)**
HIV mono- Infected	514 ± 169	28380 ± 5193
HIV/HBV Co-Infected	364 ± 181	33667 ± 5023
HIV/ HCV Co-Infected	512 ± 123	527 ± 128
HBeAg Positive	185± 10	N/A

The mean viral load was not calculated for the HBeAg positive participants because only one of them was available for the viral load assessment. *N/A=record not available*

The mean viral load was generated using viral load results of persons who had records on the ARVs they had taken. The mean viral load for the HIV mono-infected participants was lower than that of the HIV/HBV co-infected whereas the HIV/HCV co-infected participants had the lowest mean HIV-1 viral load (Table 4.5b).

From the regression analysis HBeAg positivity was associated with low CD4 count. (Mean CD4 count = 185± 10, p=0.048) (Table 8).

Table 8: A regression analysis of HBeAg positivity as a possible risk factor for reduced immunity

Participants Stratified by HBeAg status	MeanCD4 Count (cells/µl)	Regression Coefficient (95% CI)	P-Value
HBeAg Negative	500± 253	1	
HBeAg Positive	185± 10	-1.2 (-2.39 - -0.012)	0.048

4.6 Effect of HIV/HBV or HIV/HCV Co-Infection on HIV Viral Load

The regression analysis indicated that HIV/HBV or HIV/HCV co-infection did not result in an increase in the viral load of participants close to the time of delivery or at the time of delivery. However, only 9 (16%) had undetectable HIV viral load. Out of the 9 participants with undetectable HIV viral load, 7 (78%) were HIV mono-infected participants.

From the univariate regression analysis, being diagnosed of HIV during pregnancy lead to a significant increase in the HIV viral load (p= 0.045) as compared to being diagnosed of HIV before pregnancy. The multivariate analysis involving HIV/HBV co-infection and specific period during pregnancy at which the HIV was diagnosed proved otherwise (p= 0.472) (Table 9)

Table 9: The effect of HIV/HBV or HIV/HCV and timing of HIV diagnosis on HIV viral load

Parameter/Category of Participant	HIV-Viral Load			
	Univariate Regression Coefficient	**P-Value**	**Multivariate Regression Coefficient**	**P- Value**
HIV-Mono-Infected	1		1	
HIV/HCV	-35824.5	0.388	1	
HIV/HBV	-2901.7	0.896	-34453.8	0.335
Timing of HIV Diagnosis:				
Before Pregnancy	1		1	
During Pregnancy	36294.9	0.045	45091.6	0.472
Trimester in which HIV diagnosis was made				
First trimester	1		1	
Second trimester	28009	0.258	39861	0.178
Third trimester	83259	0.005	61967.7	0.009

Participants who were diagnosed of HIV in the third trimester of pregnancy had increased viral load according to both the univariate regression analysis and after a multivariate regression analysis including timing of diagnosis, HIV/HCV and HIV/HBV co- infection (Table 9).

CHAPTER FIVE

DISCUSSION

Hepatitis B and Hepatitis C infections have become a major problem for people living with HIV in an era where HAART has brought improvement into their lives (Koziel and Peters, 2007). Co-infection of these viruses is of even a greater importance in pregnancy. This is because of the risk of vertical transmission and the negative implications associated with the acquisition of these infections early in life (Toussi et al, 2007).

5.1 Prevalence of HBV and/or HCV Co-Infection

A study by Barth *et al* reviewed HBV and HCV prevalence data among HIV-infected persons in 60 studies from 18 countries in Sub-Saharan Africa including Ghana. In that study, the mean prevalence of HBsAg was 14.9% and the mean prevalence of anti-HCV was 6.9% (Barth et al, 2010). Similar to the findings of by Barth *et al*, our present study found the prevalence of HIV/HBV co-infection to be 14.9% and the prevalence of HIV/HCV co-infection to be 4.1% among HIV-infected pregnant women. Gerretti *et al* (2010) found the prevalence of HIV/HBV co-infection to be 16.7%, Sagoe *et al* (2012) found HIV/HBV and HIV/HCV co-infection to be 13% and 3.6% respectively among HIV infected persons in Ghana. These findings are also similar to the findings in our study.

The HIV/HBV co-infection prevalence of 14.9% in this study is higher than the 4.9% observed in HIV-infected pregnant women in Europe by Landes *et al* in 2008. However, Landes *et al* found HIV/HCV co-infection in HIV-infected pregnant women in Europe to be 12.3% which is higher than the 4.1% observed in our study. This is reflective of the fact that Europe has a higher prevalence of HCV as compared to sub-Saharan Africa whereas HBV prevalence is higher in Sub-Saharan Africa as compared to Europe (Alter, 2006).

This present study also found HBV prevalence to be higher (14.9%) among HIV infected pregnant women than observed in the HIV-negative pregnant women (10%). However the HIV sero-status did not have a significant effect on the HBV infection rate (p= 0.431). This is similar to the finding of Simpore *et al* (2006) in Burkina Faso (HBV prevalence of 11.6% and 7% among HIV-positive and HIV-negative pregnant women respectively, p= 0.167). Our finding is contrary to that observed by Rouet *et al* who found similar prevalence of HBV among HIV-infected and HIV-negative pregnant women in Abidjan, Côte d'Ivoire (Rouet et al, 2004).

This present study found HCV infection to be higher in the HIV-negative pregnant women as compared to their HIV-infected counterparts (12% and 4.1% respectively).This is contrary to the observation of Simpore *et al* (2006) who found HCV prevalence to be higher in HIV-positive pregnant women and Rouet *et al* (2004) who found HCV prevalence to be similar among the two groups of pregnant women. Our observation may represent an increased prevalence of HCV in the communities. However, the relatively small number of HIV-negative individuals recruited into this study (considering the prevalence of HIV among pregnant women in the Brong-Ahafo Region in 2010 to be 2.6%) (NACP, 2011), might have impacted on this outcome.

None of the participants in this study had triple infection of HIV, HBV and HCV. This is consistent with studies carried out among HIV-infected patients in South-Eastern Nigeria (Diwe, 2014) and among children on ART in Benue state Nigeria (Anigilaje and Olutola, 2013). Again, Ezechi *et al* (2014) found the prevalence of HIV/HBV and HCV triple infection among pregnant women in Nigeria to be 0.08%, suggesting that HIV/HBV/HCV triple infection is uncommon in our setting. However, Badridze *et al* (2008) found the triple infection of HIV/HBV/HCV among HIV- infected patients in Georgia to be 5.1% contrary to our finding. This is because although HIV and HBV prevalence in Sub-Saharan Africa is

high, the HCV prevalence in Sub-Saharan Africa is lower than in Europe. Again most infections of these viruses occur through the perinatal and sexual transmission in Africa. The HCV is not efficiently transmitted through these routes whereas the HCV is efficiently transmitted parenterally which is the most common mode of transmission of these viruses in Europe. This makes the possibility of triple infection of these viruses less likely in Africa as compared to the Europe (Alter, 2006).

5.2 Possible Risk Factors for Co-Infection

Factors such as the age, marital status, history of receiving blood transfusion, history of sharing sharp objects, and occupational status did not put participants at a statistically significant risk of getting HIV/HBV and HIV/HCV co-infection. Similarly, Zenebe *et al* (2014) revealed that HIV and HBV infection among pregnant women in Ethiopia was independent of marital status, occupation and age. Nimzing *et al* (2009) revealed that the occupation, marital status and history of blood transfusion did not pose a significant risk of getting HIV/HCV co-infection among HIV-infected patients. Sharing of sharps was not a risk factor for HIV/HCV co-infection among pregnant women (Duru et al, 2009).

Contrary to our finding, Mohammad *et al* **(**2009) observed that, the marital status, history of blood transfusion, occupation and age were significant risk factors for HIV/HBV and HIV/ HCV co-infections among HIV-infected patients, Zenebe *et al* (2014) found out that history of blood transfusion and sharing sharp objects increases the risk of getting infected with HBV. Again, a significant association between history of blood transfusion and HIV/HBV infection was observed by Ezechi *et al,* (2014).

The participants in this study were mainly adults. Ghana falls into a region of high HBV prevalence (CDC, 2012**)** where most HBV infection occurs through perinatal transmission or early in life through household contacts (Alter, 2006). Sexual transmission is the most

predominant mode of transmission of HIV in Ghana (UNAIDS, 2012c). These may have influenced the fact that, none of the possible risk factors we investigated posed a significant risk for co-infection of these viruses. Giving the high prevalence of HIV/HBV and HIV/HCV co-infection among HIV-infected pregnant women in this study, sexual transmission might have played a role. However we did not investigate high risk sexual behaviours among the participants.

5.3 Immunological, and Virological Characteristics and the Use of Antiretroviral Drugs

Although the WHO in 2013 recommended the start of ART when the CD4 count of HIV infected persons is ≤ 500cells/µl (WHO, 2013b), in Ghana ART for HIV infected persons was started at CD4 count ≤ 350 at the time of this study (NACP, 2008).

Whereas the majority (55.6%) of the HIV/HBV co-infected participants had their baseline CD4 count ≤ 350 cells/µl, the majority (75%) of the HIV mono-infected participant and all the HIV/HCV mono-infected participants had their CD4 count >350 cells/µl.

The mean baseline CD4 counts of the HIV/HBV co-infected participants, HIV/HCV co-infected participants and the HIV-mono-infected participants were 364 (± 181), 512 (± 123) and 514 (± 269) respectively. This means that HIV/HBV participants were more likely to be immunosuppressed as compared to the HIV- mono infected participants, but this was not statistically significant (p= 0.108). However, HBeAg positivity was associated with severe immunosuppression (mean CD4 count = 185± 10). Severe immunosuppression is defined as CD4 count < 200 cells/µl (WHO, 2007b). HIV/HCV co-infection did not significantly affect the baseline CD4 count of participants (p= 0.880).

Although we found out that the mean HIV-viral load of the HIV/HBV co-infected participants was higher whereas that of the HIV/HCV was lower than that observed in the

HIV mono-infected participants, co-infections of these viruses did not have a significant effect on the viral load of the participants (Table 4.6). This might be due to the fact that 98.2% of the pregnant women used in the analysis received (3TC+AZT+EFV or 3TC+AZT+NVP or 3TC + TDF + NVP) which are recommended first-line regimen for the treatment of HIV-infected adults including HIV-HBV co-infected persons (WHO, 2013b). Participants who were diagnosed in the third trimester were at a significant risk of having higher detectable HIV-RNA viral load as compared to persons diagnosed earlier. This might be related to the late initiation of anti-retroviral prophylaxis in such instances as the WHO recommends the immediate initiation of ARV-prophylaxis for better results (WHO, 2013b). However, we did not investigate into the timing of initiation of ARV-prophylaxis. A lower percentage (16%) of the participants analysed had undetectable viral load close to the time of delivery. This suggests that there are other factors that may affect the HIV-viral load that we did not investigate.

Sagoe *et al* (2012) observed that HIV co-infections with HBV or HCV did not affect the CD4 count and HIV-viral load among ART- naïve HIV-infected patients in Ghana. They also observed that HBeAg positivity was associated with severe immunosuppression among participants in their study. Wondimeneh *et al* (2013) also observed that HIV/HBV and HIV/HCV co-infection did not result in a significant reduction in the CD4 in HIV infected persons in Ethiopia. Our observation is comparable to these findings.

In contrast with our results, Landes *et al* (2008) observed that HIV/HBV and HIV/HCV co-infected pregnant women in Europe were significantly more immunosuppressed as compared to their HIV mono-infected counterparts. They also observed that, HIV/HCV co-infection was also significantly associated with higher HIV-RNA viral load at the time of delivery as compared to the HIV mono-infected counterparts. HIV/HCV co-infection increases lymphocyte apoptosis which leads to a decreased CD4 count (Nunez et al, 2006).

CHAPTER SIX

CONCLUSIONS

6.1 Conclusions

In conclusion the study demonstrated a high prevalence of hepatitis B virus and hepatitis C virus co-infection among HIV-infected pregnant women. HIV/HBV co-infection was found to be associated with a decreased possibility of seroconversion from hepatitis B e-antigen to antibodies to the hepatitis B e-antigen.

The study observed that the majority of the HIV diagnosis was made in the second and third trimester of pregnancy which does not help in the effort to prevent mother to child transmission of HIV. Again this study found that diagnosis of HIV in the third trimester of pregnancy was associated with detectable HIV viral load, close to the time of delivery.

The study also found that the co-infection of hepatitis B virus or hepatitis C virus and HIV did not have a significant impact on the immune status and HIV viral load. Again the study found that many of the participants had detectable HIV-viral load even with the right antiretroviral drug regimen. This suggests that factors such as non-adherence to the medication must be considered for better outcomes.

The study established that HIV/HBV or HIV/HCV co-infection was not significantly associated with the age, history of sharing sharp objects, history of blood transfusion, occupation and marital status of the pregnant women.

6.2 Recommendations

Further investigation into the prevalence of HBV and/or HCV co-infection among pregnant women should be carried out in a larger population.

The programs aimed at the prevention of HIV should be integrated with HBV and HCV preventive efforts.

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QUESTIONNAIRE

You are kindly requested to assist us fill this questionnaire. This will take about 3minutes of your time. You are not under any obligation to do so. You can choose not to answer any question that you are uncomfortable with.

Date……

Participant ID……Age……. (yrs)

Height…… (m) Weight…… (Kg)

Area of Residence……

Occupation……

Marital Status: Single □ Married □ Divorced □ Widow □

Timing of HIV diagnoses (√)

Before pregnancy□ During Pregnancy□

If you were diagnosed of HIV during pregnancy in which trimester were you diagnosed? (√) First Trimester □ Second Trimester □ Third Trimester □

Have you ever been transfused with blood? (√)

Yes □ No □

Have you ever shared sharp objects (blades, scissors etc) with others? (√)

Never □ Ever □

Table 2.6a: Recommended HIV post-exposure prophylaxis (PEP) for percutaneous injuries

	Infection status of source				
Exposure type	HIV-Positive Class 1	HIV-Positive Class 2	Source of unknown HIV status	Unknown source	HIV-Negative
Less severe	Recommend 2-drug post-exposure prophylaxis (PEP)	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP for source with HIV risk factors	Generally, no PEP warranted; however, consider basic for 2-drug PEP in settings where exposure to HIV-infected persons is likely	No PEP warranted
More severe	Recommend expanded3-drug PEP	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP for source with HIV risk factors	Generally, no PEP warranted; however, consider basic for 2-drug PEP in settings where exposure to HIV-infected persons is likely	No PEP warranted

HIV-positive class 1 describes asymptomatic HIV infection or known low viral load (e.g., <1,500 RNA copies/mL) and HIV-positive class 2 describes symptomatic HIV infection,

AIDS, acute seroconversion, or known high viral load. When the source of HIV exposure is a deceased person with no sample for testing or the source of the exposure is a needle from a sharps box, it is described as an unknown source (CDC, 2001).

Table 2.6b: Recommended HIV post-exposure prophylaxis (PEP) for mucous membrane exposures and non-intact skin exposures (CDC, 2001).

Exposure type	HIV -Positive Class 1	HIV-Positive Class 2	Source of unknown HIV status	Unknown source	HIV-Negative
Small volume	Consider basic 2-drug PEP	Recommend basic 2-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP for with HIV risk factors	Generally, no PEP warranted; however, consider basic 2-drug PEP in settings where exposure to HIV- infected persons is likely	No PEP warranted
Large volume	Recommend basic 2-drug PEP	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP for source with HIV risk factors	Generally, no PEP warranted; however, consider basic 2-drug PEP in settings where exposure to HIV-infected persons is likely	No PEP warranted

Small volume refers to few drops whereas large volume refers to major splash of body fluids. Unknown source refers to splash from inappropriately disposed body fluid. The designation, "consider PEP," indicates that PEP is optional and should be based on an individualized

decision between the exposed person and the treating clinician. If PEP is offered and taken and the source is later determined to be HIV-negative, PEP should be discontinued (CDC, 2001).

The table 2.6c gives the preferred and alternative regimen for nPEP

Preferred Regimen	• Tenofovir + Emtricitabine/Lamivudine + Raltegravir
Preferred Alternative Regimen	• Tenofovir + Emtricitabine/Lamivudine + Ritonavir-Boosted Darunavir, Atazanavir, or Fosamprenavir
Other Alternative Regimen	• Tenofovir + Emtricitabine/Lamivudine + Zidovudine • Tenofovir + Emtricitabine/Lamivudine + Lopinavir/Ritonavir • Zidovudine + Lamivudine/ Emtricitabine + one of the following ritonavir-boosted protease inhibitors: Darunavir, Atazanavir, Fosamprenavir, or Lopinavir

Table 2.6c**:** The preferred and alternative regimen for non-occupational post-exposure prophylaxis for HIV (NYSDOH, 2013).

Category of HIV infected individual	Preferred first-line regimen	Alternative first line regimens
Adults (including pregnant and breastfeeding women, TB and HBV co-infected persons	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + NVP
Adolescents (10-19 years) ≥35kg	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + NVP ABC + 3TC + EFV (or NVP)
Children from 3 years and less than 10 years. Adolescents ≤ 35kg	ABC + 3TC + EFV	ABC + 3TC + NVP AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + EFV TDF + 3TC (or FTC) + NVP
Children less than 3 years	ABC (or AZT)+ 3TC +LPV/r	ABC + 3TC + NVP AZT + 3TC + NVP

*ABC =abacavir *LPV/r =lopinavir/ritonavir

Table 2.8.1a: A summary of the first-line ART drug regimen for different groups of HIV infected persons (WHO, 2013a).

Target Population	Preferred Regimen	Alternative
Adults, adolescents (>10 years), pregnant and breastfeeding mothers	AZT + 3TC + LPV/r AZT + 3TC + ATV/r	TDF + 3TC (or FTC) + ATV/r TDF + 3TC (or FTC) + LPV/r
HIV and HBV co-infected adults	AZT + TDF + 3TC (or FTC) + (ATV/r or LPV/r)	
Children (if NNRTI-base first line regimen was used)	AZT (or ABC) + 3TC + NVP	ABC + 3TC + LPV/r TDF + 3TCor FTC+LPV/r
If PI-based first line regimen was used: Children < 3 years	No change from first line regimen in use	AZT (or ABC) + 3TC + NVP
Children (3years to less than 10 years	AZT (or ABC) + 3TC+ EFV	ABC (or TDF) + 3TC +NVP

ATV/r =atazanavir/ritonavir

Table 2.8.1b: A summary of the second line ART drug regimen for different groups of HIV infected persons (WHO, 2013a)

Drug	Dose	Activity against HIV
Interferon (INF)-α 2b	5 million units (MU) daily or 10 MU 3 times a week subcutaneous for 16-18 weeks.	Yes
PEG-INF-α 2a	180 μg once weekly subcutaneous for 48 weeks	Yes
Adefovir (ADF	10 mg x 1 daily	No
Entecavir	5.5 mg x 1 daily(1.0 mg/day if 3TC resistant)	Yes
Lamivudine (3TC	300 mg x 1 daily	Yes
Telbivudine	600 mg x 1 daily	No
Tenofovir (TDF)	300 mg x 1 daily	Yes
Emtricitabine (FTC)	200 mg x 1 daily	Yes

Table 2.8.2: A summary of drugs with activity against HBV, their dosage and whether they have activity against HIV or not (WHO, 2011).