# CO-INFECTION OF HIV AND HBV AMONG PREGNANT WOMEN ATTENDING ANTE-NATAL CARE IN SOME HOSPITALS IN KADUNA METROPOLIS, NIGERIA.

**BY**

**ABA, HENRIETTA ONEH**

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY.**

**DEPARTMENT OF MICROBIOLOGY,**
**FACULTY OF SCIENCE,**
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**(M.Sc/SCIEN/05751/2009-2010).**

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## DECLARATION

I declare that the work in this thesis, entitled "Co-infection of HIV and HBV among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis, Nigeria" has been performed by me in the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, under the supervision of Professor C.M.Z.Whong and Dr M. Aminu-Mukhtar. The information derived from the literature has been duly acknowledged in the list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

| Aba, Henrietta Oneh | _____ | _____ |
|---|---|---|
| Name | Signature | Date |

## CERTIFICATION

This thesis entitled "Coinfection of HIV and HBV among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis, Nigeria by Aba, Henrietta Oneh meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its contributions to knowledge and literary presentation.

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## DEDICATION

To Gabriel, you are an angel.

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## ABSTRACT

The transmission routes and some risk factors that are associated with human immunodeficiency virus (HIV) infection are also associated with hepatitis B virus (HBV) infection. The HIV-infected pregnant cohort represents a unique population and infection with HBV is considered a public health problem worldwide. This study was conducted to determine the sero-prevalence of HIV and HBV and co-infection of both viruses among pregnant women in Kaduna, Nigeria. Eight hundred (800) pregnant women attending ante-natal care in four selected hospitals were recruited for this study. Blood samples were collected and tested for presence of HIV antibodies and Hepatitis B surface antigen (HBsAg) using Determine HIV 1 and 2 rapid test kit and First Response antigen detection kits respectively. The positive HIV blood were retested using Unigold and analyzed for CD4 counts, while positive HBsAg plasma were confirmed using ELISA and tested for various markers of hepatitis B virus. Antibodies to HIV 1 and 2 were detected in 5.9% (47/800) of the pregnant women while HBsAg was detected in 3.9% (31/800) of the pregnant women. The prevalence of HIV and HBV co-infection was 0.5% (4/800). Mean CD4 count in the HIV positive pregnant women was 396 cells/µl of blood while a mean CD4 count of 299 cells/µl of blood was recorded for the HIV and HBV co-infected women. The predominant HBV markers detected among the HBV positive pregnant women was Anti-HBc (58.1%:18/31) while the least was Anti-HBs (3.2%:1/31). Test for markers of HBV indicated Anti-HBc as the most predominant (18:58.1%), while Anti-HBs was the least (1:3.2%). Both HBV and HIV were significantly associated with polygamy, blood transfusion and surgery ($P < 0.05$). Therefore screening for HIV and HBV during pregnancy is essential to improve ante-natal care and inform clinical management.

## TABLE OF CONTENTS


| Title | Page |
|---|---|
| Cover Page - - - - - - - - - - | i |
| Title Page - - - - - - - - - - | ii |
| Declaration - - - - - - - - - - | iii |
| Certification - - - - - - - - - - | iv |
| Dedication - - - - - - - - - - | v |
| Acknowledgements - - - - - - - - - - | vi |
| Abstract - - - - - - - - - - | vii |
| Table of Contents - - - - - - - - - | viii |
| List of Figures - - - - - - - - - - | xiii |
| List of Tables - - - - - - - - - - | xiv |
| List of Appendices - - - - - - - - - | xv |

**CHAPTER ONE**

| | |
|---|---|
| 1.0 Introduction - - - - - - - - - | 1 |
| 1.1 Statement of Research Problem - - - - - - - | 4 |
| 1.2 Justification - - - - - - - - - | 5 |
| 1.3 Aim - - - - - - - - - - | 6 |
| 1.4 Specific Objectives - - - - - - - - | 6 |
| 1.5 Research Questions - - - - - - - - | 7 |
| 1.6 Research Hypothesis - - - - - - - - | 7 |

**CHAPTER TWO**

2.0 Literature Review - - - - - - - - 8

2.1 Human Immunodeficiency Virus - - - - - - 8

2.1.1 History - - - - - - - - - - 8

2.1.2 Virology - - - - - - - - - 9

2.1.3 Immunology - - - - - - - - - 11

2.1.4 Epidemiology - - - - - - - - - 13

2.1.5 Transmission and Risk factors - - - - - - - 14

2.1.6 Pathology of HIV - - - - - - - - - 17

2.1.7 Replication and Gene expression - - - - - - 18

2.1.8 Replication of HIV - - - - - - - - 19

2.1.9 Pathogenesis of Untreated HIV Infection - - - - - 21

2.2.0 Diagnosis of HIV Infection - - - - - - - 27

2.2.1 Treatment of HIV Infection - - - - - - - 31

2.2.1.1 Overview of Antiretroviral Medication - - - - - 31

2.2.1.2 Initiating Treatment - - - - - - - - 32

2.2.1.3 Monitoring Antiretroviral Therapy - - - - - - 33

2.2.1.4 Changing Antiretroviral Therapy - - - - - - 35

2.2.1.5 Immune Modulators for HIV Treatment - - - - - 36

2.2.1.6 Prophylaxis against Opportunistic Infections - - - - 37

2.3 Hepatitis B Virus - - - - - - - - - 38

2.3.1 History - - - - - - - - - - 38

2.3.2 Virology - - - - - - - - - 38

2.3.3 Genome - - - - - - - - - 39

2.3.4 Replication - - - - - - - - - 40

2.3.5 Serotypes and Genotypes - - - - - - - 40

2.3.6 Pathogenesis - - - - - - - - - 42

2.3.7 Transmission - - - - - - - - - 44

2.3.8 Epidemiology - - - - - - - - - 45

2.3.9 Clinical Manifestation - - - - - - - - 46

2.4.0 Diagnosis - - - - - - - - - 46

2.4.1 Prevention - - - - - - - - - 48

2.4.2 Treatment - - - - - - - - - 50

2.4.3 Prognosis - - - - - - - - - 51

2.4.4 Reactivation - - - - - - - - - 51

2.5 Hepatitis B and HIV Co-infection - - - - - - 52

2.5.1 Impact of HIV on the course of HBV Infection - - - - 53

2.5.2 Diagnosis of HBV in HIV Infection - - - - - - 54

2.5.3 Management of HBV in HIV Co-infected Individuals - - - - 55

2.5.4 Prevention of HBV Transmission - - - - - - 56

2.5.5 Hepatitis B Virus Therapy - - - - - - - 56

2.5.6 Indicators for Treatment of HBV in HIV Infection - - - - 56

2.5.7 Treatment of HBV in the Setting of Antiretroviral Therapy - - - 57

2.5.8 Treatment of HBV in the Absence of Antiretroviral Therapy - - - 57

2.5.9 Hepatitis B Virus Medication - - - - - - - 58

2.6.0 Hepatitis B Virus Resistance - - - - - - - 60

2.6.1 Monitoring on Therapy - - - - - - - - 61

2.6.2 Hepato-cellular Carcinoma Screening - - - - - - 62

2.6.3 Immunization against HBV in HIV Infection - - - - - 63

**CHAPTER THREE**

3.0 Materials and Methods - - - - - - - - 64

3.1 Study Area - - - - - - - - - 64

3.2 Study Population - - - - - - - - - 64

3.3 Control - - - - - - - - - - 65

3.4 Ethical Approval - - - - - - - - - 65

3.5 Inclusion and Exclusion Criteria - - - - - - - - 65

3.6 Sample Size - - - - - - - - - 65

3.7.1 Questionnaire Administration - - - - - - - - 66

3.7.2 Specimen Collection - - - - - - - - - 66

3.8 Analysis of Specimen for HIV - - - - - - - - 66

3.8.1 HIV Screening using Determine - - - - - - - 67

3.8.2 CD4+ Count Determination - - - - - - - - 69

3.8.3 HBV Screening - - - - - - - - - 69

3.8.3.1 Antigen Detection Technique using the First Response HBsAg Card - 70

3.8.3.2 ELISA Assay - - - - - - - - - 70

3.8.4 Detection of Markers of HBV Infection - - - - - - 72

3.9 Data Analysis - - - - - - - - - 72

**CHAPTER FOUR**

4.0 Results - - - - - - - - - 74

**CHAPTER FIVE**

5.0 Discussion - - - - - - - - - 101

**CHAPTER SIX**

6.0 Conclusion and Recommendations - - - - - - 109

6.1 Conclusion - - - - - - - - - 109

6.2 Recommendations - - - - - - - - 109

6.3 Limitations of the Study - - - - - - - - 110

**REFERENCES** - - - - - - - - - 111

**APPENDICES** - - - - - - - - - 125

## LIST OF FIGURES


**Figure** **Page**

Figure 4.1 Venn diagram of the distribution of HIV, HBV and its co-infection in pregnant women attending ante-natal care in Kaduna metropolis - 75

Figure 4.2 Distribution of HIV and HBV by family type in pregnant women attending ante-natal care in Kaduna metropolis - - - - 82

Figure 4.3 Distribution of HIV and HBV by history of blood transfusion in pregnant women attending ante-natal care in Kaduna metropolis - 85

Figure 4.4 Distribution of HIV and HBV by presence of tribal mark and scarification in pregnant women attending ante-natal care in Kaduna metropolis - - - - - - - 87

Figure 4.5 Distribution of HIV and HBV by history of sexually transmitted infections in pregnant women attending ante-natal care in Kaduna metropolis - 88

Figure 4.6 Distribution of HIV and HBV by history of injury with unsterilized object such as nail cutter, nail-files, cuticle removal and razor in pregnant women attending ante-natal care in Kaduna metropolis - - - - 90

Figure 4.7 Distribution of HIV and HBV by history of surgery in pregnant women attending ante-natal care in Kaduna metropolis - - - - 92

Figure 4.8 Distribution of pregnant women with HIV and its co-infection with HBV according to CD4 count range - - - - - - 99

Figure 4.9 CD4 Count range amongst HIV seropositive pregnant women attending hospitals in Kaduna metropolis - - - - - - 100

## LIST OF TABLES


**Table** **Page**

Table 4.1 The age distribution of HIV and HBV and their co-infection among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - - - - - - 77

Table 4.2 Distribution of HIV and HBV gestational age among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - 78

Table 4.3 Distribution of HIV and HBV by educational status among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - 80

Table 4.4 Distribution of HIV and HBV by marital status among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - 81

Table 4.5 Distribution of HIV and HBV geopolitical zone among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - 84

Table 4.6 Distribution of HIV and HBV number of previous pregnancies among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - - - - - - 94

Table 4.7 Distribution of HIV and HBV number of previous sexual partners among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - - - - - - 96

Table 4.8 Distribution of HBV markers in HBsAg reactive pregnant women attending ante-natal care in some hospitals in Kaduna metropolis - - - 98

## LIST OF APPENDICES

Appendix Ia: Ethical Permit from Kaduna State Ministry of Health - - 125

Appendix Ib: Ethical Permit from St. Gerard Catholic Hospital - - - 126

Appendix Ic: Ethical Permit from 44 Nigerian Army Reference Hospital - - 127

Appendix II: Consent form - - - - - - - - 128

Appendix III: Structured Questionnaire - - - - - - 129

Appendix IV: Table showing the CD4+ of the HIV positive women - - 131

Appendix V: ELISA result of the HBsAg reactive pregnant women - - 132

# CHAPTER ONE

## 1.0 INTRODUCTION

Human immunodefficiency virus (HIV) types 1 and 2 are lentiviruses belonging to the family *Retroviridae*, and the etiologic agents of acquired immune defficiency syndrome (AIDS). The acquired immune syndrome was first described in 1981, and HIV-1 was isolated by the end of 1983 (Brooks *et al*., 2010). Human Immunodefficiency Virus attacks the body's immune system. Normally, the immune system produces white blood cells and antibodies that attack viruses and bacteria. The infection fighting cells are called T-cell lymphocytes. Months to years after a person is infected with HIV, the virus destroys all the T-cell lymphocytes. This disables the immune system to defend the body against diseases and tumors. Various infections will be able to develop, these opportunistic infections take advantage of the body's weakened immune system. These infections which normally not cause severe or fatal health problems will eventually cause the death of the HIV patient (FMOH, 2010a).

Infection with Hepatitis B virus (HBV) is a widespread problem. Epidemiological survey showed that about 5% of the world populations are asymptomatic carriers (Omer, 1995). Chronic HBV infection is a major cause of mortality and 50% of chronic carriers are expected to die from the disease due to liver cirrhosis or hepatocellular carcinoma (Omer,1995). Approximately, 350 million people are infected with HBV worldwide (Liu and Hou, 2006).

The virus has been detected in peripheral mononuclear cells, tissues of pancrease, spleen, kidney and skin, and fluids like saliva, semen, sweat, breast milk, tears, urine and vaginal secretion (Willey *et al*., 2008; Chen *et al*., 2009). Some factors affecting the prevalence of chronic HBV include age at time of infection and mode of acquisition, which vary geographically. In the United States and Western Europe, HBV often is acquired in adolescence or adulthood via sexual contact or injection drug use (Luetkemeyer, 2010). Although

spontaneous clearance of HBV acquired in adulthood occurs in over 90% of immunocompetent individuals, HIV-infected persons are half as likely as HIV-uninfected persons to spontaneously clear HBV (Luetkemeyer, 2010). Therefore, chronic HBV infection occurs in 5-10% of HIV-infected individuals who are exposed to HBV, a rate 10 times higher than that for the general population (Luetkemeyer, 2010). Hepatitis B virus is a DNA virus that forms stable circular covalently closed (ccc) DNA that can persist in the liver indefinitely (Pungpapong *et al*., 2007). Individuals with evidence of past infection (core antibody positivity) are at risk of HBV reactivation, particularly in the setting of severe immunocompromise, prolonged steroid use, or chemotherapy (Luetkemeyer, 2010).

Hepatitis B virus is classified into nine different genotypes using alphabetical nomenclature (A through I) (Firnhaber and Ive, 2010) with the coexistence of genotypes A and E in Nigeria and West Africa (Olinger *et al*., 2006), genotypes A and D predominant in South Africa. Genotype A is divided into at least 6 subgenotypes, A1 to A6. Subgenotype A1, which is found in South Africa, has unique characteristics that differentiate it from the other subgenotypes of A and genotype D. In genotype A, loss of hepatitis B e-antigen (HBeAg) cannot occur as a result of the precore stop codon mutation classically shown to cause truncation of the HBeAg precursor. In subgenotype A1, HBeAg seroconversion occurs as a result of mutations in both the basic core promoter and precore regions that affect HBeAg expression at the transcriptional, translational, and post-translational levels. These mutations may lead to loss of HBeAg expression without the expected reduction in viral loads, producing false-negative results via traditional serologic methods used for the diagnosis of actively replicating HBV (Firnhaber and Ive, 2010). Genotype G may be predictive of more severe fibrosis in HIV co-infected patients, and genotypes C and D may be more responsive to interferon (Luetkemeyer, 2010). However, in

general, knowledge of the HBV genotype is not consistently associated with a response to nucleoside therapy and therefore is not particularly useful in clinical care of HIV and HBV co-infection, as nucleosides are the mainstays of HBV treatment (Luetkemeyer, 2010). Hepatitis B virus is the leading cause of chronic liver disease and liver-related death worldwide, with the majority of these cases occurring in areas of Africa and Asia where HBV prevalence is high (Hoffmann and Thio, 2007).

Co-infection of HIV and HBV is a state in which an individual is infected with both the HIV and the HBV viruses (Omonkhelin *et al*., 2010). The risk of co-infection is not uncommon especially in areas of high prevalence and people at high risk for parenteral infection (Liu and Hou, 2006).

Co-infections with HIV and HBV is common, with 70-90% of HIV-infected individuals in the United States having evidence of past or active infection with HBV (Luetkemeyer, 2010). Many of the countries that are affected by hepatitis B are also affected by a high HIV burden, leading to frequent HIV and HBV co-infection (Hoffmann and Thio, 2007).

In the United States, HIV and HBV co-infection rates are highest among men who have sex with men (MSM) and injection drug users (Luetkemeyer, 2010). In contrast, in Asia and sub-Saharan Africa, vertical and early childhood exposure are the most common modes of transmission, respectively, there is an overall high prevalence of HBV (Luetkemeyer, 2010). The prevalence of HBV among HIV-infected individuals also is higher, at an estimated 20-30% (Luetkemeyer, 2010). The prevalence of HIV and HBV co-infection in ante-natal populations in Nigeria is as high as 8.9% (Adesina *et al*., 2010)

The consequences of co-infection, including increased liver-related morbidity and mortality, increased hepatitis B viral replication, immune reconstitution to HBV in the situation

of antiretroviral therapy, and hepatotoxicity from antiretroviral drugs, are especially important in regions with expanding antiretroviral programmes (Hoffmann and Thio, 2007). Infection with HIV not only increases the risk of persons progressing from acute infection to chronic HBV infection, but also accelerates the progression to liver disease (Puoti *et al*., 2002).

Human immunodeficiency virus and HBV have overlapping transmission routes, hence many people are infected with both viruses (Uneke *et al*., 2005). Rates of hepatitis B in the population as a whole have fallen dramatically since the advent of routine childhood HBV vaccination, but trends among people with HIV have not been well studied (Chun *et al*., 2010).

A majority of HIV negative individuals infected with HBV as adults spontaneously clear the virus without treatment, but 5-10% develop chronic infection lasting more than 6 months (Brooks *et al*., 2010). There are two main reasons for considering HBV therapy as a priority in HIV and HBV co-infected patients: Firstly, liver disease may progress more rapidly in those patients co-infected with HIV and HBV and could lead to serious liver disease complications such as cirrhosis and liver cancer at younger ages. Secondly, there is a higher risk of developing hepatotoxicity following the initiation of antiretroviral therapy in HIV patients co-infected with HBV than in patients infected with HIV alone (Highleyman, 2009).

## 1.1 Statement of Problem

Hepatitis B virus infection is a dynamic disease and co-infection with HIV impacts directly on the outcome of HBV infection, considerably complicating its natural history, diagnosis, and management (Aghasadeghi *et al.*, 2011). Few studies have addressed the issue of co-infection of HBV in HIV infected pregnant women to date, and in particular, there are few data on the prevalence of HBV and HIV co-infection in ante-natal populations (Landes *et al.*, 2008).

Many of the countries that are affected by hepatitis B are also affected by a high HIV burden, leading to frequent HIV and HBV co-infection (Hoffmann and Thio, 2007). In patients infected with HBV, HIV can lead to higher rates of chronicity, decreased rates of anti-HBe and anti-HBs seroconversion, and increased viral replication, probably due to the impairment of the body's immune responses (Hoffmann and Thio, 2007).

Human Immunodefficiency virus and HBV are both major global health concerns as they cause high mortality and morbidity in the developing countries. Due to the overlapping transmission routes; sexual contacts, blood transfusion, mother to child, use of unsterilized sharp objects such as needles, razor etc, many people are infected with both viruses (Willey *et al*., 2008). In immunocompromised pregnant women co-infection of HIV and HBV results in higher risk of maternal complications, and vertical transmission with higher rate of fetal and neonatal infections leading to liver disease that increases maternal mortality and morbidity (Elinav *et al*., 2006).

**1.2 Justification**

Whereas 40 million individuals are estimated to be infected with HIV worldwide, nearly 400 million people are chronic HBV carriers (Hoffmann and Thio, 2007). Hepatitis B virus and HIV are endemic in the same world regions, although HBV is more infectious than HIV (Hoffmann and Thio, 2007). Hepatitis B virus infection in Nigeria has remained a public health issue. It is a major cause of mortality, especially in developing countries (Eke *et al*., 2011). Vertical transmission of HBV infection is thought to be a major route of transmission in low resource areas. In spite of this, routine ante-natal screening for hepatitis B infection is not yet practiced in many Nigerian hospitals (Eke *et al*., 2011). Knowledge of the immune status of

pregnant women if known will help the clinical management of the woman and reduce vertical transmission risks.

This will also help in advising and taking precautionary measures to protect the mothers and their unborn children, and provide information required for prevention or management of HIV and HBV. Testing for HBV infection in pregnancy is important in view of the morbidity and mortality of the host (pregnant women), its effect on the process of parturition, and the risk of vertical transmission from mother-to-child (Landes *et al.*, 2008). The infected newborn most often remains a chronic carrier with the attendant consequences of liver cirrhosis, and hepatocellular carcinoma (HCC). Mother-to-child transmission can be avoided by vaccination of the newborn. This intervention to stop vertical transmission can only be applied when the status of the pregnant woman is known (Landes *et al.*, 2008). Therefore understanding the epidemiology of HBVand HIV co-infection in pregnant women is important because of vertical transmission risks and to inform clinical management (Landes *et al.*, 2008).

### 1.3 Aim

The aim of the study is to determine the prevalence of HIV and HBV co-infection and the effect on CD4+ cell count levels among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

### 1.4 Specific Objectives

1. To determine the seroprevalence of HIV and HBV and their co-infection rate among pregnant women attending ante-natal care in some selected hospitals in Kaduna metropolis.
2. To determine the effects of co-infection of HIV and HBV on the CD4+ cell count levels.
3. To determine some possible risk factors associated with HIV and HBV infection.

## 1.5 Research Question

1. What is the prevalence of HIV among pregnant women attending ante-natal care in some selected hospitals in Kaduna metropolis?
2. What is the prevalence of HBV among pregnant women attending ante-natal care in some selected hospitals in Kaduna metropolis?
3. What is the co-infection rate of HIV and HBV among the pregnant women attending ante-natal care in the selected hospitals in Kaduna metropolis?
4. What are the demographic and risk factor(s) associated with co-infection with HIV and HBV in the study population in Kaduna metropolis?

## 1.6 Research Hypothesis

Null hypothesis ($H_o$): There is no co-infection of HIV and HBV among pregnant women attending ante-natal care in some selected hospitals in Kaduna metropolis.

Alternative hypothesis ($H_a$): There is co-infection of HIV and HBV among pregnant women attending ante-natal care in some selected hospitals in Kaduna metropolis.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Human Immunodefficiency Virus

#### 2.1.1 History

Widespread awareness of HIV disease began with a brief report in 1981, published in the Morbidity and Mortality Weekly Report, of a rare pneumonia caused by *Pneumocystis carinii* (now known as *P. jiroveci*) as well as other unusual infections in 5 young homosexual men in Los Angeles (Gottlieb, 2006). Awareness that a significant epidemic was developing grew as case reports mounted and similar immune defficiency syndromes were described in New York, California, and elsewhere among homosexual men, intravenous drug users, Haitians, hemophiliacs, recipients of blood transfusions, infants, female sexual partners of infected men, prisoners, and Africans (Gottlieb, 2006).

As researchers began to describe the epidemiology and risk factors in a systematic way, many theories emerged regarding the cause of the mysterious disease (Gottlieb, 2006). An infectious agent was postulated, and, in 1983, a novel human retrovirus was isolated as the putative etiologic agent. That virus was eventually named human immunodefficiency virus, or HIV. Despite dramatic advances in basic virology and clinical management, HIV infection has developed into a worldwide pandemic, with tens of millions of individuals infected by the virus and many millions more affected by it. Clinicians treating HIV are challenged by a clinically complex illness with relatively limited resources for treatment in most settings (Gottlieb, 2006). The first case in Nigeria was reported in 1986 in a 13 year old girl. Since then, the epidemic has persisted with national HIV seroprevalence rate of 1.8% in 1991, 5.8% in 2001, 4.4% in 2005 and 4.6% in 2008 (FMOH, 2010a).

### 2.1.2 Virology

There are two HIV types, HIV type 1 (HIV-1) and type-2 (HIV-2) both of which have 60% RNA sequence homology in some regions and 30 to 40% in other regions (Sell and Max, 2001). Human Immunodefficiency virus type 1 and the less common HIV-2 belong to the family of *Retroviridae*. Human Immunodefficiency virus type 1 contains a single-stranded RNA genome that is 9 kilobase in length and contains 9 genes that encode 15 different proteins. The major viral proteins (some of which contain >1 protein subunit) are classified as structural proteins (*Gag*, *Pol*, and *Env*), regulatory proteins (*Tat* and *Rev*), and accessory proteins (*Vpu, Vpr, Vif*, and *Nef*) ( Spira *et al*., 2003).

Three major classes of HIV-1 have emerged: M (main), N (new), and O (outlier). Among M group viruses, which account for >90% of HIV infections worldwide, there are 9 subtypes, called clades, designated by the letters A-D, F-H, J, and K, as well as many recombinant forms (Spira *et al*., 2003). Variation between one clade and another in the amino acid sequences of the envelope protein may exceed 30%. Clade B, the most common subtype in the Americas and Western Europe, differs considerably from those clades found in Asia and Africa, where the majority of HIV-infected individuals reside. Viral diversity is greatest in sub-Saharan Africa. Most HIV drug development has targeted clade B. As HIV treatment is extended into regions where non-B clades predominate, issues of differential drug response, drug mutation patterns, and reliability of viral testing (ie, viral loads and resistance testing) may emerge. Sequence diversity among various clades also needs to be considered in vaccine development, as most HIV-specific neutralizing antibodies and some cytotoxic T-lymphocyte (CTL) responses are type specific (Caride *et al*., 2000; Pillay *et al*., 2000).

Human Immunodefficiency virus infection of a host cell begins with the binding of the virus particle (virion) to the host cell. This process is initiated when the surface envelope protein *Env*, (which consists of 3 copies each of the 2 subunit proteins gp120 and gp41) engages its primary receptor, the CD4 molecule on the surface of the target cell. Initial binding to CD4 exposes another portion of the *Env* trimer, which then binds to a coreceptor, usually the chemokine receptor CXCR4 (in the case of T-cell-tropic, or syncytium-inducing strains of HIV) or the chemokine receptor CCR5 (in the case of macrophage-tropic, or nonsyncytium-inducing strains). This coreceptor binding causes the gp41 trimer portion of the envelope molecule to spring open and "harpoon" the lipid bilayer of the target cell membrane. The "hairpin" domains of gp41 then fold together to pull the virus and host cell membranes together, allowing fusion to occur. The viral contents, including copies of the viral genetic material and the Pol protein (reverse transcriptase, or RT) thus enter the cytoplasm of the host cell. Reverse transcription, that is, the copying of the viral genetic material from RNA into DNA can then occur (Sell and Max, 2001).

The preintegration complex (PIC), composed of the copied DNA (cDNA) and a number of viral and host proteins, then enters the cell nucleus, where the viral enzyme integrase mediates the insertion of the viral cDNA into the host chromosomal DNA. The resulting integrated DNA virus (also called a provirus, to distinguish it from the virion form) may remain latent for hours to years before becoming active through transcription (copying of DNA into RNA). Transcription of the viral genome is under complex control of a number of proteins, including Tat and cellular DNA transcription factors. Transport of the transcribed viral RNA out of the nucleus also depends on a number of host and viral factors, including *Rev*. The transcribed viral RNA may be transported out of the nucleus in its full-length form to serve as genetic material for

new virions, or it may be partially or fully spliced. The unspliced, partially spliced, and fully spliced versions of viral RNA direct the synthesis of different viral proteins by the cell ribosomes. New viral particles are assembled at the plasma membrane and incorporate *Gag* subunits, *Pol, Nef, Env, Vpr*, and viral genomic RNA (Zimmerman *et al*., 2002).

The HIV viral protease enzyme acts following virion assembly to cleave viral proteins into functional structural and enzymatic components. *Gag* then functions in the budding of mature virions from the plasma membrane. The *Nef* protein acts on the cellular environment to promote replication by inhibiting the host immunologic response to HIV and inhibiting death of infected cells by apoptosis (Greensway *et al*., 2002).

Current HIV therapies inhibit the viral replication process at the binding and entry stage (fusion inhibitors), the reverse transcription stage (nucleoside and nonnucleoside reverse transcriptase inhibitors [NRTIs and NNRTIs, respectively]), or the protein cleavage stage (PIs). Inhibitors of coreceptor binding, integration, and maturation are in clinical trials (Willey *et al.*, 2011).

### 2.1.3 Immunology

Individuals infected with HIV show both cellular and humoral (antibody) immune responses to the virus, but these responses are unable to prevent the ultimate progression of disease in the great majority of infected individuals. Cellular responses are mediated by CTLs (CD8 cells) and helper T lymphocytes (CD4 cells). The Cytotoxic T-Lymphocytes inhibit HIV replication both directly, by recognizing and killing infected cells, and indirectly, by producing soluble chemokine antiviral factors (Brooks *et al*., 2010).

Cytotoxic T-Lymphocyte mediated killing of virally infected host cells occurs through direct contact, whereby the T-cell receptor on the surface of the CTL recognizes a fragment

(epitope) of an HIV protein bound to a major histocompatibility complex (MHC) class 1 molecule on the surface of the infected host cell. After this interaction, the CTL releases enzymes that kill the infected cell. The CTL responses directed against certain epitopes of the *Gag* protein have been associated with slower HIV disease progression than CTL responses against other epitopes. The CTLs also exert effects through soluble factors such as regulated and normal Tcell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1-alpha, and MIP-1-beta, which inhibit HIV from infecting new cells by blocking HIV coreceptors (Malnati *et al*., 2000).

The CD4 responses to HIV are important in viral control, and strong HIVspecific CD4 responses are associated with lower HIV viral loads. The CD4 cells respond to HIV antigens presented in conjunction with MHC class II molecules on the surface of infected cells. The fact that HIV infects CD4 cells themselves is an evolutionary strategy with a number of consequences. Because productive HIV infection occurs in activated CD4 cells, infection and killing of CD4 cells that are responding to HIV infection itself may cause a selective decrease in the number of HIV-specific CD4 cells. HIV can also exist in nonactivated CD4 cells in a preintegrated form, which can become integrated if activation occurs within a few days (McMicheal and Rowland-Jones, 2001).

Additionally, as some of the activated, infected CD4 cells differentiate into resting memory CD4 cells, they may carry copies of the HIV genome in a postintegrated form that can persist for decades. Current antiretroviral medications cannot efficiently eliminate the virus from cells in the resting state, leading to persistence of infection even in the presence of suppressive therapy. Moreover, HIV continues to evolve under the selection pressure of the immune response that occurs in each infected individual, and mutations in the viral epitopes recognized by the

immune system may enable the virus to escape the control of even broad and robust CD4 and CD8 HIV-specific responses (McMicheal and Rowland-Jones, 2001).

Depletion of CD4 lymphocytes is the hallmark of HIV infection, and predicts an individual's risk for infection with opportunistic pathogens as well as other complications of HIV disease. Evidence has shown that both increased peripheral destruction and decreased production of CD4 cells likely play a role in this decline (Mohri *et al*., 2001).

Humoral immunity appears to be less effective in controlling viremia than cellular responses, as HIV is remarkably effective at evading host antibody responses, and broadly neutralizing antibodies are rare. The difficulty in eliciting broadly neutralizing antibody responses against HIV has posed a particularly difficult challenge to the development of a protective HIV vaccine (Peterlin *et al*., 2003).

### 2.1.4 Epidemiology

The Federal Ministry of Health, Nigeria (2009) estimates that the people living with HIV is 33.4 million worldwide of which adults were 31.3 million and women, 15.7 million, while children below 15 years were 2.1 million. The prevalence of HIV in women receiving ante-natal care was 4.6% (FMOH, 2009). The most heavily affected area of the world is sub-Saharan Africa, with approximately 67.6% of the total number of people living with HIV, 69.2% of the 2.6 million new infections and 72.2% of the 1.8 million deaths in 2009 (FMOH, 2010a). Over 90% of infection in children is acquired through mother to child transmission and as more women contract the virus, the number of children infected has been growing. In Nigeria, heterosexual transmission accounts for nearly 80% while mother to child transmission and use of unsterilized sharps, infected blood and blood products accounted for 10% each (FMOH, 2010a).

The epidemic varies by geographic region. Based on the 2007 data; sub-Saharan Africa had the highest number of HIV infections. In certain high prevalence cities in Africa, one of every three adults is infected with the virus (Brooks *et al*., 2010). The world health organization estimates that of the 2.7 million new infections each year, 90% are occurring in developing countries. In these countries, AIDS is overwhelmningly a heterosexually transmitted disease, and there are equal numbers of male and female cases (Brooks *et al*., 2010).

In countries with the highest prevalence such as Botswana and Zimbabwe, rates of HIV infection may exceed 30% in the general population and may be greater than 50% among selected groups, including pregnant women, male patients at sexually transmitted infection clinics, and female sex workers. Southeast Asia is estimated to have 6,000,000 infections, and China and the former Soviet Union are estimated to have more than 1,000,000 infections each. Infection rates are increasing in China, India, and Eastern Europe, fueled by high rates of intravenous drug use, increasing prevalence of other sexually transmitted diseases, and public health systems that are poorly prepared to prevent the spread of HIV (Hammers *et al*., 2003; Kaufmann *et al*., 2003).

### 2.1.5 Transmission and Risk Factors

The Nigerian Federal Ministry of Health (2009) report indicates that the common mode of HIV transmission are unprotected sex, transfusion of blood that is infected with HIV and use of needles or syringes contaminated with HIV infected blood. Any cut with sharp instrument contaminated with HIV infected blood especially in hospital setting is a means by which individuals especially healthcare providers can be infected. An adult or child may become

infected if they have a skin wound which comes into contact with body fluids or blood from a person who is HIV infected (FMOH, 2010a).

Human Immunodefficiency virus is believed to be transmitted by passage of viable infected blood lymphocytes or macrophages, such as transfusion of infected blood, by sharing of contaminated needles by intravenous drug users, or by passage of infected lymphocytes or macrophages in semen or cervical secretions during sexual contact through openings or breaks in the skin or mucous membranes or by direct passage of the virus through the mucous membranes of the genital tract (Sell and Max, 2001).

The primary method of spread of HIV infection worldwide is through sexual exposure (Brooks *et al*., 2010). In the United States and Europe, acquisition of the virus through homosexual contact remains important, and there is some evidence of increasing incidence of infection among young gay men and ethnic minorities. Men who have sex with men, however, now account for less than 50% of new infections in the United States. In the areas of highest HIV prevalence globally, heterosexual intercourse is the primary mode of transmission, accounting for approximately 70% of the overall sexual transmission (Gayle, 2000).

Human Immunodefficiency virus has been isolated from blood, seminal fluid, pre-ejaculatory fluid, vaginal secretions, cerebrospinal fluid, saliva, tears, and breast milk of infected individuals (Brooks *et al*., 2010). In genital fluids, HIV may be found in both cell-free and cell-associated compartments, but it is unknown which is responsible for productive infection. Viral concentrations in tears and saliva are comparatively low, and there are substances in saliva that appear to inhibit infectivity. No cases of HIV infection have been documented to arise from contact with non bloody saliva or tears. Transmission of HIV occurs more frequently through penile-anal intercourse and penile-vaginal intercourse, although clear cases of transmission

through oral sex exist (Quinn *et al*., 2000). Female-to-female HIV transmission has been reported, but is rare. In a meta-analysis, the overall efficacy of condoms in reducing HIV transmission was 69%. Sexual activity that is associated with exposure to infected blood increases the risk of transmission, as does the presence of genital ulcers. Serum HIV viral load is strongly associated with heterosexual transmission between HIV-serodiscordant African sexual partners, where transmission was noted to be rare at viral loads <1,500 copies/mL (Quinn *et al*., 2000).

The effect of viral load reduction with ART on HIV transmission is being investigated. Intervention with antiretroviral medications soon after high-risk sexual exposures has been proven to be safe and may be effective in preventing transmission of HIV (Kahn *et al*., 2001) Nonsexual HIV transmission can occur through transfusion with contaminated blood products, injection drug use, occupational exposure, or accidental needlesticks. The risk from occupational needlesticks to health care workers from known HIV-positive source patients in case series performed prior to the availability of potent ART was found to be 0.33-0.5% (FMOH, 2010a). Factors increasing the risk of HIV acquisition from an occupational needlestick include deep injury, injury with a visibly bloody device, or injury with a device that had been previously used in the source patient's vein or artery. Postexposure prophylaxis (PEP) has been associated with a reduction of HIV transmission after occupational needlestick events of approximately 80% (FMOH, 2010a).

Human Immunodefficiency virus transmission through transfusion of contaminated blood products was recognized early in the epidemic. With current testing methods, the risk of acquiring HIV from a unit of transfused blood in the United States is 1 in 500,000, but is significantly higher in many developing countries (Brooks *et al*., 2010).

In the absence of interventions, mother-to-child transmission occurs in approximately 25% of live births to HIV-infected mothers. Various regimens of antiretrovirals can reduce the rate of perinatal transmission by 50% or more (Lallemant *et al*., 2000). Breast-feeding is also a risk factor for HIV transmission. Approximately one-third of cases of mother-to-child transmission result from breast-feeding, and the risk increases with the duration of breast-feeding. Thus, interventions to prevent mother-to-child transmission at delivery may be largely negated if mothers are not provided with safe alternatives to breast-feeding (Richardson *et al*., 2003; Brooks *et al*., 2010).

### 2.1.6 Pathology of HIV

Human Immunodefficiency virus damages the immune system, leaving the infected person vulnerable to a variety of infections (called "opportunistic" infections to indicate that they arise in the setting of immune impairment). The effect of HIV on the immune system is monitored by measuring the CD4 (helper) lymphocyte count in the blood. A normal CD4 count (between approximately 600 and 1200 cells/μL) indicates that the immune system has not undergone sufficient damage to put the individual at risk for opportunistic illness. However, recent studies have demonstrated that even those with CD4 counts above 350-500 cells/μL are at elevated risk for a number of conditions that were not previously recognized as related to HIV infection (FMOH, 2010a). These include cardiovascular disease, kidney and liver disease, malignancies, and neurocognitive decline (FMOH, 2010a). As a result, experts have increasingly recommended initiation of treatment at higher CD4 counts. In persons with CD4 counts <500 cells/μL, this indicate that impairment of immune function is present, and is an indication for ART. Cluster of Differenciation 4 (CD4) counts <200 cells/μL indicate imminent risk of serious

opportunistic infections or other complications of HIV disease, and prompt treatment is recommended (Dybul *et al*., 2002). Untreated HIV disease is chronic and progressive. Primary HIV infection, often marked by a mononucleosis-like acute viral syndrome, is followed by a period of clinical latency typically lasting several years, during which high levels of viral replication and CD4 cell turnover lead to progressive immune dysfunction, eventually resulting in clinical disease progression. The progression of HIV infection is determined by the relationship of the rate of reproduction of virus to the production of new CD4 T cells (Brooks *et al.*, 2010). During latency, it is estimated that 100 billion new viral particles are being produced every day and about 2 billion CD4 cells are dying and being regenerated everyday (Sell and Max, 2001; Brooks *et al*., 2010).

### 2.1.7 HIV Replication and Gene expression

Human Immunodefficiency virus has sets of transactivators and cis-acting factors controlling transcription.The two major products that regulate viral gene expression are Tat and Rev.

**Tat:** acts as a highly effective transactivator of HIV long terminal repeat (LTR)-dependent transcription by increasing initiation or promoting elongation of transcripts. The two tat coding exons are joined by RNA splicing to produce a transcript that encodes the 14-kDa Tat protein. The Tat protein activates transcription by binding to an RNA stem-loop structure called the transactivating response element (TAR). A cellular protein kinase, Cdk9, binds to the activation domain of Tat and can phosphorylate the COOH-terminal domain of RNA polymerase II. The transactivating effects of Tat may cause proliferation of other cells (Sell and Max, 2001).

**Rev:** is a 19kDa protein encoded by RNA sequences which overlap those of tat but are in a different reading frame. Rev binds to a cis-acting RNA target, the rev-responsive region, which is a 204-nucleotide sequence in the env region of HIV. Rev acts post transcriptionally on HIV RNA transcripts and targets them for a nuclear export in concert with a cellular protein called expotin 1(Sell and Max, 2001).

**Vpr:** is an accessory/regulatory protein that delivers viral RNA to the nucleus, inhibits proliferation of HIV-infected cells, causing them to delay for extended periods in the $G_2$ phase of the cell cycle during which HIV replication takes place, and then inducing apoptosis of infected T cells, allowing release of virus particles (Sell and Max, 2001).

**Nef:** acts together with vpr to downregulate the expression of cell surface molecules, such as CD4. Nef causes increased exocytosis of CD4, and vpr targets CD4 to the endoplastic reticulum and for proteolysis by recruitment into the ubiquitin-proteasome pathway (Sell and Max, 2001).

### 2.1.8 Replication of HIV

The mature virion forms a sphere with 72 spikes that contain the Env glycoproteins. A region on the viral envelops gp120 first binds to a domain on CD4 on the lymphocyte surface. This interaction causes a conformational change in the gp120, resulting in binding of another domain onthe virus to CCR5 (R5 variant) or CXCR4 (X4) on the cell surface. Progression of latent infection to AIDS is often associated with a switch R5 to X4 variants in a given patient, but AIDS can clearly develop without this switch (Sell and Max, 2001).

The ways of subversion of the immune defence mechanisms by HIV is as follows:

**Immune evasion**: A critical factor in the progression of HIV infection is the way that HIV manages to subvert or avoid immune defence mechanisms. During HIV infection, there is

continous production of virus; billions of virions are produced every day. These in turn, infect and kill billions of CD4 cells which must be replaced to maintain immune function (Sell and Max, 2001).

**Immune exhaustion**: The enormous numbers of HIV virions produced may overwhelm the immune system. With the onset of clinical symptoms, there is a decrease in antibody titres and numbers of CD8 T cells (Sell and Max, 2001).

**Integration of HIV DNA into CD4 T cells**: In resting infected cells, HIV RNA has been reverse transcribed and integrated into the DNA of the host cells. In this site, it is not available for reactivity to antibody or T cytotoxic cells (Sell and Max, 2001).

**Destruction of CD4 T cells**: The CD4 cells are the helper cells for the immune responses and effector cells for delayed type hypersensitivy.With the loss of these functions as a result of HIV infection, the immune response to both HIV and other infections is impaired (Sell and Max, 2001).

**Inaccessibility of HIV surface antigens**: Antibodies that react with soluble HIV gp120 or gp40 may not react with these glycoproteins on the virion. The virion capsid is extensively glycosylated, covering up the potential reactive antigens on the virus. On budding from the infected cell surface, the capsid may incorporate host cell surface molecules and in this way mask viral antigens (Sell and Max, 2001).

**Downregulation of MHC**: Viral products, such as Nef, decrease expression of MHC class 1 on infected cells. Since CD8 cells recognize cell surface antigens complexed with MHC 1 molecules, loss of MHC 1 results in decreased recognition of HIV infected cells by CD8 T cytotoxic cells (Sell and Max, 2001).

**Loss of CD8 T cytotoxic cells**: Production of CD8 T cytotoxic cells requires of CD4 T cell help. With the loss of this help, the numbers of CD8 T cytotoxic cells decrease (Sell and Max, 2001).

**Loss of non cytotoxic CD8cells**: Perhaps the most important effector mechanism in HIV infection is that mediated by non cytotoxic CD8 cells. Individuals with repeated exposure to HIV, who nevertheless do not develop infection, have a strong CD8 non cytotoxic anti-HIV response. These CD8 cells inhibit HIV replication in CD4 cells, without the requirement for MHC class 1 recognition and without killing the infected cells (Sell and Max, 2001).

### 2.1.9 Pathogenesis of Untreated HIV infection

**Primary HIV infection**: Primary HIV infection is defined as the time period from initial infection with HIV to the development of an antibody response detectable by standard tests. Data from careful prospective evaluations of populations at risk for HIV infection demonstrate that up to 87% of individuals who acquire HIV may experience some symptoms of primary HIV infection (FMOH, 2010a). The acute viral syndrome of primary HIV infection (sometimes referred to as "seroconversion illness") was first defined in 1985, with symptoms resembling those of mononucleosis appearing within days to weeks following exposure to HIV. Symptoms may be mild or severe and may last from a few days to several weeks, with the average duration being 14 days. The most common presenting symptom is fever, seen in over 75% of patients. Other commonly reported symptoms include fatigue, lymphadenopathy, headache, and rash (FMOH, 2010a). The rash, which is present in 40-80% of cases, may be evanescent, typically maculopapular in character, and typically involves the trunk (FMOH, 2010a). Evaluation of cohorts from Kenya and India found more frequent reports of joint pains, night sweats, and mucosal candidiasis and less frequent rash and pharyngitis in these study populations. A more

severe clinical syndrome in primary HIV infection has been associated with a more rapid subsequent clinical course of HIV disease (Lavrey *et al*., 2000).

The nonspecific symptoms of primary HIV infection may make diagnosis a challenge. In a study of high-risk individuals presenting with symptoms consistent with primary HIV infection, only 25% were diagnosed during their initial presentation. Diagnosis of HIV during the acute seroconversion phase requires not only high clinical suspicion but also an understanding of appropriate testing strategies. Routine HIV antibody testing may be negative for several weeks or even months after exposure in the so-called "window period". During primary infection with HIV, plasma viral load often reaches very high levels in the range of millions of RNA copies/mL. Thus, for individuals in whom primary HIV infection is clinically suspected, HIV RNA assays, which have a sensitivity approaching 100% and specificity of 97.4% in this setting should be included in the diagnostic evaluation (Hetch *et al*., 2002). Human Immunodefficiency virus RNA tests are not licensed for the diagnosis of HIV infection, and positive RNA tests during acute infection should be confirmed by documentation of subsequent HIV antibody conversion. The high levels of viremia seen in primary infection do not persist, however, providing evidence of a host immune response capable of bringing the infection under some degree of control, at least in the short term (Hecht *et al*., 2002).

The CD4 counts and CD4 function may decline during primary HIV infection, occasionally to levels that allow opportunistic infections (OIs) to develop. Absolute CD4 count often rebounds after the primary infection, but may not return to a normal baseline. In patients with clinical progression of HIV disease, CD4 responses against HIV itself appear to remain particularly impaired following primary infection (Sell and Max, 2001).

After the initial reduction of viremia, a viral "set-point" is established in each infected individual. The magnitude of this set-point correlates with the rate of progression of HIV disease. Studies of individuals during primary HIV infection have raised the question of whether the set-point might be reduced by early treatment (Rosenberg *et al*., 2000). Although early antiretroviral therapy may preserve immune function rapid control of viremia may also inhibit the full development of a mature immunologic response (Oxenius *et al*., 2000; Stranford *et al*., 2001). Carefully supervised interruptions of antiretroviral treatment after initial control during acute infection may permit the development of an effective immune response in the short term (Rosenberg *et al*., 2000) but long-term follow-up suggests that increases in viral load and emergence of drug resistance may occur in such patients (Tremblay *et al*., 2003).

**Chronic HIV infection**: After the period of acute HIV infection (during which CD4 counts and viral load change dramatically), a relative equilibrium between viral replication and the host immune response is reached, and individuals may have little or no clinical manifestations of HIV infection. This time between initial infection and the development of AIDS may be long, averaging 10 years, even in the absence of treatment. Despite the relative clinical latency of this stage of HIV infection, viral replication and CD4 cell turnover remain active, with millions of CD4 cells and billions of virions produced and destroyed each day. During this period, most infected individuals will have progressive loss of CD4 lymphocytes and perturbation of immune function. On average, CD4 counts will drop by 50-90 cells/μL per year in asymptomatic individuals, usually with an acceleration of this rate over time (Sell and Max, 2001).

The rate of progression of infection may vary considerably. In adults, progression from infection to clinical AIDS is rare in the first 2 years of infection; however, reports describe rapid disease progression in infants infected by blood transfusion. In a well-characterized cohort of

HIV seroconverters who were identified in a retrospective analysis of stored serum specimens from hepatitis B vaccine trials in the 1970s, 87% of infected individuals had developed AIDS by 17 years postseroconversion. Twelve percent maintained a CD4 count >500 cells/μL at 10 years, but only 3% maintained a CD4 count >500 cells/μL at 16 years after seroconversion. During chronic HIV infection, HIV RNA levels in plasma correlate with the rate of CD4 decline, with higher plasma viral loads predicting more rapid progression to AIDS and death. An undetectable HIV RNA level in peripheral blood is associated with stable CD4 lymphocyte counts, and increases in HIV RNA correlate with more rapid rates of CD4 cell decline (Sell and Max, 2001).

**Clinical AIDS:** According to CDC criteria, AIDS is defined by either diagnosis of one of the AIDS-defining conditions, or by measurement of CD4 levels <200 cells/μL. Progression to AIDS from time of infection occurs, on average, 2 years earlier when defined by laboratory criteria (CD4 levels <200 cells/μL) compared to clinical criteria (development of an opportunistic illness). Survival time from the development of AIDS varies according to the AIDS-defining event (FMOH, 2010b).

**Host Factors**: A number of host factors influence HIV disease progression. Individuals who acquire HIV at an older age tend to have more rapid disease progression and shorter survival times. Variation in HIV coreceptor molecules, notably CCR5, influences both HIV susceptibility and disease progression. A mutant allele of CCR5 with a 32-base-pair deletion, CCR5-delta-32, is frequent in populations of European origin (10-15% of Caucasians are heterozygous, and 1% are homozygous), and encodes a nonfunctional truncated protein that is not transported to the cell surface. Homozygotes for the delta-32 allele exhibit a strong, although not complete, resistance to HIV infection, whereas heterozygotes display nearly normal rates of infection, but delayed progression to AIDS (Willey *et al*., 2008).

Genetic differences in HLA alleles have also been shown to influence HIV disease susceptibility and disease progression. The class I alleles B35 and Cw4 have been associated with accelerated progression of disease, as has general HLA homozygosity. Because HLA class I alleles determine which viral epitopes can be presented to CD8 cells, greater diversity of HLA (heterozygosity) in an individual may reflect greater options for effective cell-mediated immunity to HIV. Conversely, HLA B27 and B57 have been associated with long-term nonprogression of HIV disease. In particular, HLA B*5701 has been found to be highly overrepresented in long-term nonprogressors (Willey *et al*., 2008).

Behavioural or psychological host factors may also influence HIV disease progression. More rapid HIV disease progression has been reported with unprotected anal intercourse, smoking, poor nutrition, and depression; however, not all studies confirm these findings. Drug use might be expected to influence HIV disease progression, but studies of that question have produced mixed results. Additionally, differences in disease course based on the route of HIV transmissions have been difficult to prove (FMOH, 2010a).

**Viral Factors**: Human Immunodefficiency virus virions infect human cells by first binding to the CD4 receptor on the cell surface. This alone is not sufficient for the virus to enter the host cell; binding to an additional coreceptor is also required. Macrophage- or M-tropic viruses preferentially infect monocytes and macrophages, using the cell surface protein CCR5 (R5) as the preferred coreceptor to enter cells, and produce a nonsyncytium-inducing (NSI) phenotype in cell culture. Conversely, thymocyte- or T-tropic viruses preferentially infect T cells, use CXCR4 (X4) as the preferred coreceptor to enter cells, and produce a syncytium-inducing (SI) phenotype in cell culture. Dual-tropic viruses, which may use either CCR5 or CXCR4 coreceptors, also

exist. M-tropic viruses are frequently found in early HIV infection, and a switch to T-tropic strains in the course of disease is associated with rapid CD4 cell depletion (Brooks *et al*., 2010). The concept of viral "fitness" refers to the pathogenicity of certain strains of HIV. The HIV replicative capacity (RC) has been studied as a component of viral fitness. The term replicative capacity is a measure of the ability of a given virus to replicate successfully in a given environment. During the course of drug treatment, mutations arise in the HIV reverse transcriptase and protease enzymes that make the virus resistant to particular drugs, thus conferring a selective advantage to that subpopulation that arises from a resistant variant. Several of these mutations have been shown to cause a reduction in RC in the absence of drug when compared to wild-type virus. Further accumulation of mutations over time under drug selection pressure may increase the "fitness" of the drug-resistant variant by further increasing phenotypic resistance, or by increasing RC of the resistant virus. Other viral factors may be important as well. For example, faster rates of disease progression have been observed in Ugandan individuals infected with subtype D compared with subtype A isolates. Additionally, rare individuals who are infected with variant HIV strains, particularly those with a defective nef gene product, may experience slower disease progression (Kaleebu *et al*., 2002).

**Co-infections**: The development of opportunistic infections (OIs) during HIV disease not only indicates the degree of immunosuppression, but may also influence disease progression itself. When stratified by CD4 counts, patients with prior histories of OIs have higher mortality rates than those without prior histories of OIs. Hepatitis C co-infection is common in HIV-infected patients, present in up to 40-50% of all patients in urban setting and in 90% of intravenous drug users (Sulkowski *et al*., 2002; Willey *et al*., 2008). Human Immunodefficiency virus clearly leads to more rapid HCV disease progression; however, the effect of HCV infection on HIV

progression is less clear. In a study of the Swiss HIV Cohort, HCV co-infection was associated with poorer CD4 responses to ART, development of new AIDS-defining events, and increased mortality (Greub *et al*., 2000).

### 2.2.0 Diagnosis of HIV Infection

Laboratory diagnosis of HIV is based on the demonstration of antibodies in plasma or serum and of virus in the blood. The virus can be demonstrated in the blood with nucleic acid based tests (PCR for proviral DNA and RT-PCR for plasma viral RNA), culture and p24 antigen assay. With the technology that is available at present, HIV antibodies are detectable within 4-6 weeks of infection and within 24 weeks in virtually all infected individuals (FMOH, 2010a).

**Antibody assays**: The antibody assays that are used for HIV diagnosis consist of screening tests: rapid tests or ELISA, and confirmatory tests: western blot and indirect immunofliorescent assay. Routine antibody testing is performed with the serial or parallel testing algotithm using rapid or ELISA test (FMOH, 2010a).

**Enzyme immunoassay (EIA) for HIV**: Enzyme Immunoassay is commonly used as a screening assay for many infectious diseases, including HIV. These assays are used because they are highly sensitive and generally amenable to automation, facilitating high-volume testing. Human Immunodefficiency virus EIAs have become increasingly more sensitive and specific since HIV testing began in the early 1980s. This has shortened the 'window period', or the time from exposure to seroconversion, from up to 12 weeks or more in the early days of diagnostic testing to the current 'window period' of less than three weeks in most cases (Zhang and Versalovic, 2002; Fearon, 2005).

The small disadvantage of such a highly sensitive test is that the test produces false positives, the number and type of which vary with the assay used and the HIV prevalence in the tested population. All HIV diagnostic laboratories must confirm repeated EIA screen-positive results by a confirmatory assay, usually with Western blot (Zhang and Versalovic, 2002). Laboratories may choose to first test with a second EIA assay, which uses a different part of the viral antigen for antibody capture, as part of their testing algorithm. Specimens that are positive in the first assay but negative in the second assay should still be considered for confirmatory testing if the patient is symptomatic or high risk (Zhang and Versalovic, 2002).

**P24 Antigen**: The P24 antigen tests are also EIA-based and use antibody to capture the disrupted p24 antigen from patient serum. Positive results that are repeatable must be confirmed with a neutralization procedure. In rare instances, the p24 antigen can be detected before HIV antibody in newly infected individuals. This test is useful for specimens from patients that are high risk and symptomatic but HIV EIA-negative, or for specimens that are EIA-positive but Western blot-negative or -indeterminate. A follow-up HIV antibody test should be requested when a patient is p24 antigen-positive but antibody-negative. In a seroconverting patient, the follow-up specimen will be positive within a few weeks after the initial screen. It is important to remember that not all seroconverting patients will have detectable p24 antigen, and that this antigen may not be reliably found in individuals who are known to be HIV antibody-positive (Zhang and Versalovic, 2002; Fearon, 2005; FMOH, 2010a).

**Western Blot**: The Western blot is an immunoblot that allows for the characterization of antibodies to each viral protein. Patient serum is reacted with a nitrocellulose strip containing all of the constitutive HIV virus proteins (core and envelope), arranged by molecular weight after polyacrylamide gel electrophoresis. Any specific antibodies present in the patient's serum will

bind to the antigen, producing a coloured band when alkaline phosphatase-labelled, antihuman immunoglobulin G conjugate and colour development solution are added. These bands can be visualized, and positivity is assessed following the manufacturer's recommendations and based on the number and type of bands present. Generally, a specimen must show a positive reaction with a minimum of one core band and one envelope band to be judged positive by Western blot (Zhang and Versalovic, 2002; Fearon, 2005; FMOH, 2010a).

Specimens that have bands present but do not fulfill the criteria for positivity are called Western blot indeterminate, and a follow-up specimen should be requested, usually collected three to four weeks after the initial specimen. In follow-up, patients will either show a definitive pattern indicating that they have seroconverted or will demonstrate the same banding pattern as previously observed. In the latter circumstance, the vast majority of these patients is HIV-negative and have nonspecific antibody. In these cases, if the patient is considered to be at risk or is particularly anxious, a qualitative PCR may be recommended to confirm that the patient is truly HIV-negative. Because these indeterminate banding patterns may be seen in patients who are not infected, the Western blot does not make a good screening test for HIV. The test is also much more labour intensive and costly than EIA tests and does not allow for the efficient processing of large numbers of specimens (Zhang and Versalovic, 2002).

Specimens that have an unusual band pattern not reflective of EIA optical density values should either be tested on an HIV-2 blot or forwarded to the National Laboratory for HIV Reference Services (Ottawa, Ontario) for HIV-2 testing. Generally, HIV-2 positive specimens exhibit antibody to many HIV-specific bands but lack antibody to the envelope components of HIV-1 (Zhang and Versalovic, 2002; Fearon, 2005).

**Qualitative PCR**: Polymerase chain reaction is a method that amplifies viral nucleic acid to allow for its detection in patient specimens. It is a particularly specific and sensitive test which can pick up very small numbers of viral particles. Polymerase chain reaction is very useful in the diagnosis of HIV infection in babies born to infected mothers. Babies will carry maternal antibody up to approximately 15 months of age and, therefore, the antibody test is not a reliable indicator of infection in these children (Zhang and Versalovic, 2002).

Because of the very specific nature of the primers used in the HIV-1 DNA PCR assay, caution should be used in assessing a negative result in an infant if the mother is likely to have HIV-2 or a non-B HIV infection. It may be prudent to ensure that the HIV DNA PCR assay is effective in detecting the mother's HIV infection if there is any likelihood that the case is not an HIV subtype B infection. In North America, this would be a very uncommon finding (Zhang and Versalovic, 2002). Polymerase chain reaction may also be useful in resolving indeterminate Western blot results and testing immunocompromised individuals who may not mount an antibody response (Zhang and Versalovic, 2002; Fearon, 2005).

**Quantitative RNA PCR and Genotyping**: Quantitative RNA PCR must only be used to monitor HIV-positive individuals before or during antiretroviral therapy. It is used in conjunction with CD4 counts and general clinical assessments to ascertain when therapy should be started. It is also used to help determine the patient's response to therapy. Genotyping is used to monitor the development or presence of drug resistance in patients before or during therapy. It is also used to assist physicians in their choice of antiretroviral drug combinations for the patient (Zhang and Versalovic, 2002).

**Virus Isolation**: Human immunodefficiency virus is usually isolated in peripheral blood mononuclear cells (PBMCs). The procedure involves cocultivating the PMBCs from an HIV

positive patient with those obtained from a healthy donor. Human immunodefficiency virus isolation in PMBC is quite sensitive and is comparable to DNA PCR in sensitivity (FMOH, 2010a).

### 2.2.1 Treatment of HIV infection

#### 2.2.1.1 Overview of Antiretroviral Medications

Currently, all FDA-approved antiretroviral medications work by inhibiting 1 of 3 steps in the life cycle of HIV:

1. Blocking the reverse transcriptase (RT) enzyme. The RT enzyme is used by the virus to convert its RNA into DNA after the virus enters the cell but before it enters the nucleus. All nucleoside and nucleotide analogues as well as NNRTIs function by interfering with the activity of this enzyme (Kaplan *et al*., 2002).
2. Blocking the protease enzyme. Protease inhibitors, as their name indicates, inhibit the action of the HIV protease, namely, cleaving protein products of the viral structural genes into the functional subunits needed to create new infectious virions (Kaplan *et al*., 2002).
3. Inhibiting fusion of the viral and host membranes. By attaching itself to the HIV envelope glycoprotein gp41, fusion inhibitors prevent formation of the "hairpin" structure required for fusion of the HIV and host cell membranes, and thus prevent viral entry into the host cell (Kaplan *et al*., 2002).

New antiretroviral medications that are in development include improved formulations of currently approved drugs (to enhance bioavailability, increase half-life, or reduce adverse effects); new drugs in the same classes as currently approved drugs (such as PIs or NNRTIs with

fewer adverse effects or unique resistance patterns); and drugs with novel mechanisms of action (eg, integrase inhibitors, entry inhibitors, and HIV coreceptor blockers) (Kaplan *et al*., 2002).

#### 2.2.1.2 Initiating Treatment

Not all HIV-infected individuals require ART at a given point in time. For those whose CD4 count and clinical assessment indicate a low risk of imminent disease progression, the potential adverse effects of immediate treatment would be expected to outweigh any benefit. Others may have psychosocial barriers to adherence that preclude effective ART, at least in the short term, until conditions related to these issues can be improved (eg, through stable housing, substance abuse counseling, or treatment of medical or psychiatric conditions). Because incomplete adherence can rapidly lead to lasting resistance against available antiretrovirals, it is usually worthwhile to address issues that are likely to impair adherence before starting ART. Still other individuals may opt for complimentary/alternative medicine (CAM) for management of their disease. In one large population-based study, 3% of individuals with HIV chose to use CAM as a substitute for standard therapy (Hsiao *et al*., 2003).

Individuals for whom initiation of ART is not indicated must be monitored closely for changes in immune status (eg CD4 counts) that might signal increased risk of OIs and thus trigger initiation of ART, OI prophylaxis, or other intervention. In general, determination of CD4 counts and viral loads should be made every 3-6 months in individuals with stable infection. Measurements of CD4 count may be transiently decreased and measurements of HIV viral load transiently increased during acute infection with another pathogen or immediately after vaccination, and providers should avoid measurement of these parameters in such situations (Kaplan *et al*., 2002).

The decision to initiate (or reinitiate) ART should be made with clear goals that address the concerns of the patient as well as the provider. Active viral replication in the presence of antiretroviral drugs can be expected to result in selection of drug-resistant virus leading ultimately to treatment failure. Therefore, any antiretroviral regimen should ideally be prescribed with the intention of complete viral suppression. When complete suppression is not possible, secondary goals of treatment may include partial virologic suppression (which might be the best attainable goal if the virus is highly resistant to antiretrovirals), immunologic reconstitution, or alleviation of symptoms (such as fever, night sweats, or weight loss). Sufficient patient education on the antiretroviral regimen chosen dosing schedule, meal restrictions, anticipated adverse effects and adverse effect managementis also crucial to the success of any treatment regimen. Individual adherence is one of the most challenging and most important aspects of treatment success (Wahl *et al*., 2000; Paterson *et al*., 2000).

#### 2.2.1.3 Monitoring ART

The efficacy of an antiretroviral regimen should be monitored by regular determinations of HIV viral load and CD4 count. The first indication of successful treatment is a decline in viral load. The decline in viral load is biphasic. The first (fast) decay phase occurs during the first 2 weeks of treatment for most patients; a second (slow) phase of decay is evident thereafter. The first phase may result primarily from antiviral effects of medications, whereas immune factors such as CTL antiviral activity may contribute to the elimination of virally infected cells in the second phase. The kinetics of response are variable among individuals, and do not appear to be entirely determined by adherence or drug levels. Moreover, although viral decay rates are not entirely predictive of subsequent virologic failure, virologic response at 4 weeks of treatment has been shown to correlate with response at 48 weeks. As a rule of thumb, in a successful regimen

the viral load should decline by at least 1 log by 4 weeks after treatment initiation; slower rates should prompt closer assessment of patient adherence, viral resistance, and possible drug-drug interactions. Even a successful regimen, however, may take 4-6 months or even longer to attain undetectable viral loads (Jenny-Avital *et al.*, 2002).

As viral load declines, the number of circulating CD4 cells begins to increase. Initial increases in CD4 count during the first 1-3 months of therapy are believed to be caused primarily by a redistribution of cells trapped from lymphoid tissue. The subsequent rate of improvement in CD4 levels is variable among individuals, and gradual CD4 count increases may continue for many years with therapy that effectively suppresses HIV replication. Individuals with lower CD4 counts may have a slower and less complete CD4 recovery, whereas those who start therapy with higher CD4 counts and maintain continued viral suppression with antiretroviral medications may reach CD4 counts similar to those of HIV-uninfected individuals (Jenny-Avital *et al.*, 2002).

As the immune system begins to reconstitute itself in the early phases of ART, it may mount vigorous inflammatory responses against certain pathogens. The restoration of immune function may therefore cause paradoxical worsening of certain coexisting OIs, a phenomenon known as "immune reconstitution syndrome." Infections for which immune reconstitution syndrome has been reported include pneumocystosis, cryptococcosis cytomegalovirus (CMV) retinitis *Mycobacterium avium* complex infection, and tuberculosis. Immune reconstitution syndrome generally presents as inflammation of tissues involved in the infection (eg, marked lymphadenopathy in tuberculosis, potentially sight-threatening inflammation of the vitreous in CMV retinitis), presumably reflecting localized antigen load. The syndrome appears to occur most frequently in individuals with low CD4 counts and develops in the initial weeks of ART. It may present a diagnostic challenge to the clinician, and should be evaluated thoroughly to

exclude other etiologies such as failure of therapy or other infections. Exact diagnostic criteria and guidelines for management of immune reconstitution syndrome are being developed. No clear guidelines for the management of immune reconstitution syndrome exist; however some approaches include using systemic corticosteroid treatment, or stopping antiretrovirals in the most severe cases (Koval *et al*., 2002; Jenny-Avital *et al.*, 2002; Mutimer *et al*., 2002).

#### 2.2.1.4 Changing ART

Antiretroviral regimens may fail to suppress viral replication for a number of reasons, including incomplete adherence, poor absorption, toxicity leading to missed or lowered doses, pharmacokinetic interaction, suboptimal antiviral potency, and preexisting drug resistance. Virologic failure has been reported in as many as 63% of patients in population-based studies. However, virologic outcomes may be improving over time; in a large cohort study, 72% of subjects on therapy attained HIV RNA <500 copies/mL at 6 months (Sternert *et al*., 2003).

At the time of changing antiretroviral regimens, careful assessment of the reasons for changing should be undertaken. Understanding the factors of adherence, toxicity, and resistance that prompted the switch will aid in designing subsequent regimens and optimizing patient outcomes. Changes to regimens for reasons of toxicity may be as simple as exchanging a new drug for the one that was causing the toxicity, provided that virus resistant to the other drugs in the regimen has not emerged. Patients who have failed multiple regimens or who have highly drug-resistant virus may require a more complicated "salvage" regimen. Such regimens may contain more drugs, may involve more complicated dosing schedules and may include experimental agents. Complete viral suppression may not be an achievable goal in some patients, decisions to change or continue treatment should take into account immunologic and clinical

stability, as well as the observation that selective pressure by antiretrovirals can maintain drug-resistant mutations that impair viral replicative cycle (Niihuis *et al*., 2001; Deeks *et al*., 2003).

#### 2.2.1.5 Immune Modulators for HIV Treatment

Control of HIV viremia depends on host immune response as well as exogenous intervention with antiretroviral medications. In an effort to enhance host immune response to HIV, several techniques of immune modulation have been studied. Early attempts at immune modulation through bone marrow transplantation, donor lymphocyte infusions, and cytokine infusions demonstrated no consistent, durable clinical benefit. Immunomodulatory approaches may prove more effective in combination with effective ART (Hecht *et al*., 2002).

In the context of established infection, therapeutic immunization has been studied as a means to enhance HIV-specific immune responses. No study to date has demonstrated sustained clinical benefit to such "therapeutic vaccination." Some studies have demonstrated improved HIV-specific immune responses after therapeutic vaccination, but the clinical significance of these responses remains unclear (Hecht *et al*., 2002).

Interleukin-2, a cytokine released by activated CD4 cells that regulates T-cell proliferation and maturation, has been studied as an immune modulator in HIV-infected patients. Administration of IL-2 to individuals with controlled HIV viremia leads to increased CD4 counts, expansion of both memory and naive CD4 pools, and decreased T-cell activation (Hecht *et al*., 2002).

Techniques of immune manipulation through infusion of antigen-presenting cells that have been activated *in vitro* (Lu *et al*., 2003), infusion of expanded or activated (Levine *et al*., 2002) CD4 cells, and genetic manipulation of cells to induce anti-HIV CTL activity are also

under investigation, but even if such resource-intensive, individualized approaches are shown to be effective, it is doubtful that they will become accessible to most people infected with HIV. Administration of human growth hormone (hGH) as a method of enhancing CD4 recovery in HIV 1s another technique being studied, spurred by the observations of increased circulating naive CD4 cells and increased thymic mass, suggesting increased thymopoeisis, during hGH treatment (Napolitano *et al*., 2002; Hecht *et al*., 2002).

#### 2.2.1.6 Prophylaxis against Opportunistic Infections

Many commonly encountered OIs may be prevented by administering prophylactic antibiotics to those at risk, based on pathogen-specific CD4 cell count thresholds. In geographic areas where local epidemiology of opportunistic pathogens varies from that of the United States, different recommendations for primary prophylaxis (treatment to prevent an initial episode of an OI) may be appropriate. In general, secondary prophylaxis (treatment to prevent recurrence of an OI) should be provided as long as immune impairment persists (Kaplan *et al*., 2002).

Among individuals who experience reconstitution of immunologic function with ART, as assessed by sustained increases in CD4 count to levels above those associated with OI, discontinuation of primary prophylaxis or secondary prophylaxis has been shown to be safe (Kirk *et al*., 2002).

## 2.3 Hepatitis B Virus

### 2.3.1 History

Hepatitis B infection (serum hepatitis) is caused by the hepatitis B virus (HBV). Hepatitis B virus is a hepadnavirus—*hepa* from *hepatotrophic* and *dna* because it is a DNA virus (Brooks *et al*., 2010) and it has a circular genome composed of double-stranded DNA. The virus replicate through an RNA intermediate form by reverse transcription, and in this respect they are similar to retroviruses (Willey *et al.*, 2011). Although replication takes place in the liver, the virus spreads to the blood where virus-specific proteins and their corresponding antibodies are found in infected people. Blood tests for these proteins and antibodies are used to diagnose the infection (Brooks *et al*., 2010).

### 2.3.2 Virology

Hepatitis B virus is a member of the *Hepadnaviridae* family (Brooks *et al*., 2010). The virus particle, (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Locarnini, 2004). The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses, with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies. The outer surface contains HBV and surrounds a 27nm innercapsid core that contains HBcAg (Brooks *et al*., 2010).

### 2.3.3 Genome

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand) (Kay and Zoulim, 2007; Brooks *et al*., 2010). The negative-sense, (non-coding), is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (-) sense strand and the ends are rejoined.

There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. Hepatitis B envelop antigen is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBV). The HBV gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced (Beck and Nassal, 2007). The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules (Li *et al*., 2010).

### 2.3.4 Replication

The life cycle of hepatitis B virus is complex. Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process. The virus gains entry into the cell by binding to an unknown receptor on the surface of the cell and enters it by endocytosis. Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies.The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity (Beck and Nassal, 2007; Bruss, 2007; Brooks *et al*., 2010).

### 2.3.5 Serotypes and Genotypes

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into nine genotypes (A-I) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination (Kramvis *et al*., 2005; Firnhaber and Ive, 2010).

Genotypes differ by at least 8% of their sequence and were first reported in 1988 when six were initially described (A-F) , Two further types have since been described (G and H) (Shibayima *et al*., 2005). Most genotypes are now divided into subgenotypes with distinct properties (Schaefer, 2007).

Genotype A is most commonly found in the Americas, Africa, India and Western Europe and South-Africa. Genotype B is most commonly found in Asia and the United States. Genotype B1 dominates in Japan, B2 in China and Vietnam while B3 confined to Indonesia. Genotype B4 is confined to Vietnam. All these strains specify the serotype ayw1. Genotype B5 is most common in the Philippines. Genotype C is most common in Asia and the United States. Subgenotype C1 is common in Japan, Korea and China. Genotype C2 is common in China, South-East Asia and Bangladesh and C3 in Oceania. All these strains specify the serotype adrq. Genotype C4 specifying ayw3 is found in Aborigines from Australia (Kurbanov *et al*., 2010). Genotype D is most commonly found in Southern Europe, India and the United States and has been divided into 8 subtypes (D1-D8). In Turkey genotype D is also the most common type.

A pattern of defined geographical distribution is less evident with D1-D4 where these subgenotypes are widely spread within Europe, Africa and Asia. This may be due to their divergence having occurred before than of genotypes B and C. D4 appears to be the oldest split and is still the dominating subgenotype of D in Oceania. Type E is most commonly found in West and Southern Africa. Type F is most commonly found in Central and South America and has been divided into two subgroups (F1 and F2). Genotype G has an insertion of 36 nucleotides in the core gene and is found in France and the United States (Stuyver *et al*., 2000). Type H is most commonly found in Central and South America and California in United States. Africa has five genotypes (A-E). Of these the predominant genotypes are A in Kenya, B and D in Egypt, D

in Tunisia, A-D in South Africa and E in Nigeria (Kurbanov *et al*., 2010). Genotype H is probably split off from genotype F within the New World (Arauz-Ruiz *et al*., 2003).

### 2.3.6 Pathogenesis

Hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. The receptor is not yet known, though there is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D (Shuping *et al.*,1999; Glebe and Urban, 2007). Hepatitis B virus virions (DANE particle) bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. PreS and IgA receptors are accused of this interaction. Hepatitis B virus-preS specific receptors are primarily expressed on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells (Coffin *et al*., 2011).

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. Cytotoxic T lymphocytes eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes (Iannacone *et al*., 2007). Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Iannacone *et al*., 2005).

There are 3 main phases of chronic HBV disease as follows:

1. **Immune tolerant phase:** This phase is characterized by a high level of HBV replication with little or no evidence of active hepatic inflammation. Hepatic transaminases are normal and liver biopsy, if performed, would show little or no inflammation. Most children infected at birth or during early childhood will be immune tolerant and remain in the immune tolerant phase for years or even decades; however, most children infected will eventually progress to immune active disease. Given the lack of immune response to HBV, treatment is not recommended during this period, but individuals should be monitored for transition to immune active disease as well as for hepatic fibrosis and hepatocellular carcinoma (HCC) as they age. HBV DNA is detectable, as are HBeAg and hepatitis B surface antigen (HBV). Because of the high level of HBV viremia, this is a highly infectious phase (Richman *et al.*, 2002).

2. **Immune active phase:** This phase is characterized by elevated liver enzymes and liver inflammation on biopsy. Individuals infected in adolescence or adulthood, as is frequently seen in HIV and HBV co-infection in the United States, often begin the disease course in the immune active phase, and are never immune tolerant. This phase also is characterized by detectable HBV DNA, HBeAg and HBV, and risk of transmission to others (Richman *et al.*, 2002).

3. **Inactive carrier phase:** During this phase, HBeAg is lost and HBV DNA declines, often to undetectable levels. Hepatitis B e antibody (HBeAb) seroconversion can occur, followed by hepatitis B surface antibody (HBsAb) seroconversion, indicating immune control of HBV infection. A small proportion of people will continue to have detectable low-level HBV DNA, which may be intermittent and is referred to as "occult" HBV infection (Richman *et al.*, 2002).

HBeAg-Negative Chronic Hepatitis*:*The "e antigen negative" chronic hepatitis is a form of immune active hepatitis in which individuals have undergone loss of eAg and eAb seroconversion but still have HBV DNA present at fluctuating levels and evidence of active

hepatitis with hepatic inflammation. eAg is not detectable because the HBV has undergone mutations in the precore or basal core promoter region, resulting in little to no eAg production. Despite the lower levels of HBV viremia, eAg-negative hepatitis can be difficult to treat, usually requiring indefinite treatment with nucleoside therapy, rarely transitions to inactive carrier phase, and is associated with a high risk of progression to fibrosis. Both the eAg-negative and the inactive carrier phases are characterized by core antibody (cAb)-positive, eAb-positive, eAg-negative, and sAg-positive serologies. They are distinguished by detection of HBV DNA, measured at serial time points (every 3 months); evidence of detectable HBV DNA is suggestive of eAg-negative chronic hepatitis. In addition, eAg-negative disease is characterized by ongoing liver inflammation and, in most cases, elevated transaminases.

Resolved infection*:* HBV-infected individuals who have lost e Ag and sAg and seroconverted to eAb and sAb positivity are referred to as having resolved their HBV infection. However, given the nature of the long-lived cccDNA that resides in hepatocytes during HBV infection, it may be more appropriate to think of these individuals as immunologically controlled because, in rare cases, HBV disease can reactivate in the setting of severe immunosuppression (Richman *et al.*, 2002).

**2.3.7 Transmission**

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood such as saliva, nasopharyngeal washings, semen, menstrual fluid and vaginal secretions. Possible forms of transmission include sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for HBV confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also

positive for HBeAg. Hepatitis B virus can be transmitted between family members within households from carriers, possibly by contact of non intact skin or mucous membrane with secretions or saliva containing HBV by oral route or by sexual or intimate exposure. However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor as patients and staff of hemodialysis units are exposed to contracting the infection (Brooks *et al*., 2010).

**2.3.8 Epidemiology**

Hepatitis B is worldwide in distribution. Transmission modes and response to infection vary, depending on the age at the time of infection (Brooks *et al*., 2010). In 2004, an estimated 350 million individuals were infected worldwide. National and regional prevalence ranges from over 10% in Asia to under 0.5% in the United States and northern Europe. Routes of infection include vertical transmission (such as through childbirth), early life horizontal transmission (bites, lesions, and sanitary habits), and adult horizontal transmission (sexual contact, intravenous drug use) (Custer *et al*., 2004). The primary method of transmission reflects the prevalence of chronic HBV infection in a given area. In low prevalence areas such as the continental United States and Western Europe, injection drug abuse and unprotected sex are the primary methods, although other factors may also be important (Redd *et al*., 2007). In moderate prevalence areas, which include Eastern Europe, Russia, and Japan, where 2–7% of the population is chronically infected, the disease is predominantly spread among children. In high prevalence areas such as China and South East Asia, transmission during childbirth is most common, although in other areas of high endemicity such as Africa, transmission during childhood is a significant factor (Alter, 2003). The prevalence of chronic HBV infection in areas of high endemicity is at least 8%. As of 2010, China has 120 million infected people, followed

by India and Indonesia with 40 million and 12 million respectively. According to WHO, an estimated 600,000 people die every year related to the infection (Willey *et al*., 2011).

### 2.3.9 Clinical Manifestation

Acute infection with HBV is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few patients may have more severe liver disease (fulminant hepatic failure), and may die as a result of it. The infection may be entirely asymptomatic and may go unrecognized (Terraut *et al*., 2005).

Chronic infection with HBV may be either asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of Membranous glomerulonephritis (MGN) (Gan *et al*., 2005).

### 2.4.0 Diagnosis

Current serologic assays for the diagnosis of acute and chronic HBV infection (HBV and HBeAg) are both sensitive and specific. Hepatitis B surface antigen, anti-HB core, and anti-HBe are detected by standardized enzyme-linked immunoassays. The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. It is the first

detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or HBcAg. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (anti-HBc IgM) may be the only serological evidence of disease (Brooks *et al*., 2010; Willey *et al*., 2011).

Shortly after the appearance of the HBV, another antigen named as the hepatitis B e antigen (HBeAg) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (anti-HBe) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication (Brooks *et al*., 2010; Willey *et al*., 2011).

If the host is able to clear the infection, eventually the HBV will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen, (anti-HBs and anti HBc IgG). The time between the removal of the HBV and the appearance of anti-HBs is called the window period. A person negative for HBV but positive for anti-HBs has either cleared an infection or has been vaccinated previously (Brooks *et al*., 2010; Willey *et al*., 2011). Individuals who remain HBV positive for at least six months are considered to be hepatitis B carriers (Lok and Mcmahon, 2007; Brooks *et al*., 2010; Willey *et al*., 2011). Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, as revealed by biopsy. Carriers

who have seroconverted to HBeAg negative status, particularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others (Chu and Liaw, 2007).

Polymerase chain reaction tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim, 2006; Brooks *et al*., 2010; Willey *et al*., 2011). Individuals with high viral loads, characteristically have ground glass hepatocytes on biopsy (Brooks *et al*., 2010).

### 2.4.1 Prevention

Several vaccines have been developed for the prevention of HBV infection. These rely on the use of one of the viral envelope proteins (hepatitis B surface antigen or HBV). The vaccine was originally prepared from plasma obtained from patients who had long-standing hepatitis B virus infection. However, currently, it is made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine (Brooks *et al*., 2010; Willey *et al*., 2011).

The risk of vertical transmission to the newborn can be drastically reduced from 20%-90% to 5%-10% by administering to the newborn hepatitis B vaccine (HBV 1) and hepatitis B immune globulin (HBIG) within 12 hours of birth, followed by a second dose of hepatitis B vaccine (HBV 2) at 1-2 months and a third dose at and no earlier than 6 months (24 weeks). Since 2% of infants vaccinated will not develop immunity after the first three dose series, infants born to hepatitis B positive mothers are tested at 9 months for hepatitis B surface antigen (HBV) and the antibody to the hepatitis B surface antigen (anti-HBs); if post-vaccination test results

indicate that the child is still susceptible, a second three dose series at (0, 1 and 6 months) is administered. If the child is still susceptible after the second series, a third series is not recommended (Brooks *et al*., 2010; Willey *et al*., 2011).

Following vaccination, hepatitis B surface antigen may be detected in serum for several days; this is known as vaccine antigenaemia (Martin-Ancel *et al*., 2004). The vaccine is administered in two-, three-, or four-dose schedules into infants and adults, which provides protection for 85–90% of individuals. Protection has been observed to last 12 years in individuals who show adequate initial response to the primary course of vaccinations, and that immunity is predicted to last at least 25 years (Vandamme and Van Herck, 2007; Brooks *et al*., 2010; Willey *et al*., 2011).

Hepatitis B does not generally spread through water and food like hepatitis A. Instead, it is transmitted through blood and body fluids such as saliva, sweat, semen, breast milk, urine, feces; prevention is thus the avoidance of such transmission: unprotected sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission during child birth (Willey *et al.*, 2011). Infants may be vaccinated at birth as the virus can pass through the placenta to the fetus (Willey *et al.*, 2011).

Shi *et al.*(2010) showed that besides the WHO recommended joint immunoprophylaxis starting from the newborn, multiple injections of small doses of hepatitis B immune globulin (HBIg, 200–400 IU per month) (Li *et al*., 2004 ; Shi *et al*., 2010), or oral lamivudine (100 mg per day) in HBV carrier mothers with a high degree of infectiousness (>$10^6$ copies/ml) in late pregnancy (the last three months of pregnancy) (Li *et al*., 2004 ; Shi *et al*., 2010), effectively and safely prevent HBV intrauterine transmission, which provide new insight into prevention of HBV at the earliest stage.

### 2.4.2 Treatment

Acute hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously (Hollinger and Lau, 2006). Early antiviral treatment may only be required in less than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Lai and Yuen, 2007).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. Currently, there are seven medications licensed for treatment of hepatitis B infection in the United States. These include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude) and the two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys). The use of interferon, which requires injections daily or thrice weekly, has been supplanted by long-acting PEGylatedinterferon, which is injected only once weekly (Dienstag, 2008). However, some individuals are much more likely to respond than others and this might be because of the genotype of the infecting virus or the patient's heredity. The treatment reduces viral replication in the liver, thereby reducing the viral load (the amount of virus particles as measured in the blood) (Pramoolsinsup, 2002).

Infants born to mothers known to carry hepatitis B can be treated with antibodies to the hepatitis B virus (HBIg). When given with the vaccine within twelve hours of birth, the risk of acquiring hepatitis B is reduced 90%. This treatment allows a mother to safely breastfeed her child (Libbus and Phillips, 2009).

Response to treatment differs between the genotypes. Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases. Sustained e antigen loss after treatment is ~45% in types A and B but only 25–30% in types C and D (Cao, 2009).In July 2005, researchers identified an association between a host-derived DNA-binding protein and the amount of HBV replication in the liver. Controlling the level of production of this protein could be used to treat hepatitis B (Ng *et al*., 2005).

### 2.4.3 Prognosis

Hepatitis B virus infection may either be acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months. Children are less likely than adults to clear the infection. More than 95% of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, this drop to 30% for younger children and only 5% of newborns that acquire the infection from their mother at birth will clear the infection (Bell and Nguyen, 2009). This population has a 40% lifetime risk of death from cirrhosis or hepatocellular carcinoma (Dienstag, 2008). Of those infected between the ages of one to six, 70% will clear the infection (Kerkar, 2005).

### 2.4.4 Reactivation

Hepatitis B virus DNA persists in the body after infection and in some people the disease recurs (Vierling, 2007). Although rare, reactivation is seen most often in people with impaired immunity (Katz *et al*., 2008).

### 2.5.0 Hepatitis B and HIV Co-infection

Markers of HBV exposure are present in a high proportion of HIV-infected individuals. Human immunodefficiency virus affects HBV viral replication and clearance, accelerates the development of liver disease, and contributes significantly to hepatic morbidity and mortality in HIV infection (Hoffmann and Thio, 2007).

Co-infection with HBV and HIV is common, with 70-90% of HIV-infected individuals in the United States having evidence of past or active infection with HBV (Rodriguez-Mendez *et al*., 2000; Hoffmann and Thio, 2007). Factors affecting the prevalence of chronic HBV include age at time of infection and mode of acquisition, which vary geographically (Hoffmann and Thio, 2007). In the United States and Western Europe, HBV often is acquired in adolescence or adulthood via sexual contact or injection drug use. Although spontaneous clearance of HBV acquired in adulthood occurs in >90% of immunocompetent individuals, HIV-infected persons are half as likely as HIV-uninfected persons to spontaneously clear HBV. Therefore, chronic HBV infection occurs in 5-10% of HIV-infected individuals who are exposed to HBV, a rate 10 times higher than that for the general population (Alter *et al*., 2003). In the United States, HIV and HBV co-infection rates are highest among men who have sex with men (MSM) and injection drug users. In contrast, in Asia and sub-Saharan Africa, where vertical and early childhood exposure are the most common modes of transmission, respectively, and overall HBV prevalence is higher, the prevalence of HBV among HIV-infected individuals also is higher, at an estimated 20-30% (Uneke *et al*., 2005; Hoffmann and Thio, 2007).

Human immunodefficiency virus and HBV co-infection increases HIV and HBV replication, hepatitis flares, and risk of progression to chronic HBV infection, cirrhosis, and hepatocellular carcinoma. Human immunodefficiency virus and HBV co-infection decreases

frequency of hepatitis B e antibody (anti-HBe) and hepatitis B surface antibody (anti-HBs) seroconversion, increases risk of antiretroviral therapy-related hepatotoxicity, and reduces efficacy of HBV therapy. All newly diagnosed HIV patients should be screened for hepatitis A, B, and C viruses and vaccinated if not immune to hepatitis A or B viruses. Hepatitis B virus serology often is atypical in co-infection. Diagnosis of HBV co-infection in HIV infection is made on the basis of hepatitis B surface antigen (HBV)-positive, hepatitis B core antibody (anti-HBc total)-positive, and anti-HBs-positive status. Alanine aminotransferase levels in co-infected patients often are not reliable markers of liver inflammation. Hepatitis B virus infection should always be treated if co-infected patients are receiving antiretroviral therapy, since immune reconstitution under antiretroviral therapy poses risk for immune-associated liver damage in these patients (Peter, 2007).

### 2.5.1 Impact of HIV on the Course of HBV Infection

The course of acute HBV may be modified in the presence of HIV infection, with a lower incidence of icteric illness and lower rates of spontaneous clearance of HBV (Piroth *et al*., 2007). Persons with HIV and chronic HBV co-infection have higher levels of HBV DNA and lower rates of clearance of the hepatitis B envelop antigen (HBeAg). Serum transaminase levels may be lower in HIV and HBV co-infected patients than in HBV-monoinfected patients, but normal transaminase levels should not be interpreted to mean that there is no underlying hepatic fibrosis (Piroth *et al*., 2007).

Human immunodefficiency virus increases the risk of cirrhosis and end-stage liver disease in HBV co-infection (Thio *et al*., 2002). Liver-related disease has emerged as the leading cause of non-HIV-related mortality in parts of the world where effective antiretroviral therapy

(ART) is widely available. In several cohort studies, the risk of liver-related mortality has been found to be 2-3 times higher in HIV and HBV-co-infected patients than in HIV-monoinfected patients (14% vs 6%). Lastly, HIV co-infection is associated with more frequent flares of hepatic transaminases, which can occur with immune reconstitution inflammatory syndrome (IRIS) owing to ART, interruption of HIV and HBV treatment, or the development of resistance to HIV and HBV treatment; they also can occur spontaneously (Bonacini *et al*., 2004; Weber *et al*., 2006).

### 2.5.2 Diagnosis of HBV in HIV infection

Given the elevated rates of HBV among individuals who are infected with HIV and the shared routes of transmission between the two viruses, all HIV-infected individuals should be screened for HBV co-infection with HBV testing. Patients who do not have evidence of HBV should have HBcAb and HBsAb evaluated to assess for prior HBV infection as well as HBsAb, which provides evidence of immunologic control of prior infection or vaccination. Because HBV DNA can persist in the liver indefinitely, persons with cAb positivity are at risk of HBV reactivation even if they test positive for sAb, particularly if they become severely immunosuppressed (Firnhaber *et al*., 2009).

**Isolated HBcAb**: Some individuals will have isolated HBcAb positivity, in the absence of HBV or HBsAb. That indicates one of the following scenarios:

1. **Occult" HBV Viremia:** HBV viremia may be detected in 4-88% of isolated cAb-positive patients. The clinical significance of occult viremia is unknown. However, as even low levels of HBV viremia may increase the risk of liver disease these individuals may be at higher risk of

hepatic disease and many experts recommend managing these patients as chronically HBV infected (Gandhi *et al*., 2005; Iloeje *et al*., 2007; Firnhaber *et al*., 2009; Luetkemeyer, 2010).

2. **Waned HBsAb Response:** Loss of anti-HBs can occur over time, particularly with immunosuppressed patients. In the absence of detectable HBV viremia, these individuals may respond to a single booster HBV vaccination. However, the rate of anamnestic response after 1 booster is low (7-16% in 2 studies of isolated cAb-positive patients) suggesting that it may be more efficient to complete a 3-vaccine series before checking sAb titer to determine response (Luetkemeyer, 2010).

3. **False-positive HBcAb:** Individuals who do not respond to a booster of HBV vaccination may have a false-positive HBcAb result or may have an impaired antibody response to prior infection, therefore vaccination with a full series is recommended.

Persons with detectable sAg should be evaluated for HBeAg, HBV DNA viral load, liver transaminases, prothrombin time/international normalized ratio (PT/INR), and platelets and they should have baseline liver imaging to screen for cirrhosis and HCC (Firnhaber *et al*., 2009; Luetkemeyer, 2010).

### 2.5.3 Management of HBV in HIV Co-infected Individuals

Patients with chronic HBV should be screened for hepatitis C virus (HCV) infection and should be vaccinated against hepatitis A virus (HAV) if they are not immune. HIV and HBV-co-infected patients should be counseled to avoid or limit intake of hepatotoxins, including alcohol and high dosages of acetaminophen (Firnhaber *et al*., 2009; Luetkemeyer, 2010).

### 2.5.4 Prevention of HBV Transmission

All HBV-positive patients should be counseled about reducing the risk of HBV transmission to close contacts. It is important to inform HIV and HBV-co-infected patients that HBV can be more infectious than HIV and can be transmitted to household contacts via dried blood, open cuts, and shared toothbrushes or razors. Sex partners, household members, children with close physical contact, and those who share injection drug equipment with the patient should be screened for HBV and vaccinated if they are not actively infected. As with HIV prevention, condom use with sex and avoidance of shared needles and other equipment for injection drug use are recommended measures for reducing the risk of HBV transmission (Benhamou, 2006).

### 2.5.5 Hepatitis B Virus Therapy

The goal of HBV treatment for persons with HIV co-infection is to suppress HBV viral replication and minimize ongoing hepatic damage. Loss of surface antigen (sAg) and seroconversion to sAb indicating resolution of active HBV disease are uncommon in HIV and HBV co-infection; therefore, indefinite treatment of HBV for co-infected patients often is required (Benhamou, 2006).

### 2.5.6 Indicators for Treatment of HBV in HIV infection

Hepatitis B virus therapy is recommended for all HIV and HBV-co-infected patients with abnormal alanine aminotransferase (ALT) values or HBV DNA levels of >2,000 IU/mL. Some guidelines cite >20,000 IU/mL as the threshold for treatment of eAg-positive patients, however, many experts recommend treatment of HBV for all HIV-co-infected patients in whom any HBV

replication is present. If HBV viremia is low level (<2,000 IU/mL) and the ALT value is normal, a liver biopsy may be considered for patients who are not on HIV therapy, as those with no or limited fibrosis may not require HBV treatment. It is important to note that significant liver fibrosis may be present in persons with normal transaminases. If HBV treatment is deferred, transaminases and HBV DNA values should be monitored closely (Benhamou, 2006).

### 2.5.7 Treatment of HBV in the Setting of ART

As most HIV treatment contains one or more HBV-active agents (eg, lamivudine [3TC], emtricitabine [FTC], tenofovir [TDF]), patients with an indication for HBV treatment should be started on fully active ART that contains HBV-active nucleoside/nucleotide analogues, regardless of the CD4 cell count, to ensure that HIV is not partially treated. Similarly, any HIV and HBV-co-infected patient with an indication for ART should be started on HIV treatment that includes effective anti-HBV treatment. The combination of TDF with 3TC or FTC is recommended as a highly effective first-line treatment for HBV. Individuals who cannot take TDF because of renal insufficiency or other intolerance may consider entecavir treatment (renally dosed if necessary) for HBV in lieu of TDF. Treatment with 3TC or FTC as the only HBV-active agent in ART (ie, HBV monotherapy) is not recommended owing to a high risk of developing HBV drug resistance over time (Benhamou *et al*., 1999).

### 2.5.8 Treatment of HBV in the Absence of ART

The current U.S. Department of Health and Human Services guidelines recommend fully active ART for HIV and HBV-co-infected patients who require HBV treatment, even if they do not have a current indication for HIV treatment. However, in some circumstances, an individual

may not tolerate HIV therapy or may wish to defer treatment for HIV. Treatment with HIV and HBV-active agents that are insufficient to fully suppress HIV should be avoided. Entecavir and telbivudine have been shown to have anti-HIV activity and to contribute to HIV drug resistance as have traditional HIV-active agents used for HBV treatment (eg, 3TC, FTC, TDF). Whereas adefovir may be an option for treatment of HBV only, there is a concern that even low-dose adefovir used for HBV could have anti-HIV activity. Pegylated interferon (IFN) is an option in this scenario, but there are limited efficacy and safety data in HIV co-infection, and long-term treatment with IFN is not feasible because of toxicity and poor tolerability (McMahon *et al*., 2007).

### 2.5.9 Hepatitis B Virus Medication

**Interferon**: Interferon (IFN) is most effective for HBV treatment in patients with low levels of viremia and elevated transaminases, and it therefore may be less useful in patients with HIV and HBV co-infection than in those with HBV alone. In co-infected patients, Interferon has been associated with lower rates of HBV treatment success and increased toxicity. It cannot be used for patients with decompensated cirrhosis and is not feasible as a long-term treatment, owing to adverse events and tolerability issues. There are no data for use of pegylated IFN in HIV and HBV co-infection (Di Martino *et al*., 2002).

**Lamivudine and emtricitabine**: These nucleoside analogues have similar activity against both HIV and HBV and they are commonly used components for HIV and HBV co-treatment. However, HIV-infected individuals should not receive 3TC or FTC monotherapy for HBV infection because resistance to those drugs develops in up to 90% of patients within 4 years of single-drug treatment. Resistance to 3TC and FTC is characterized by the development of

mutations at HBV rtM204 (also known as YMDD mutations). Once 3TC resistance has developed, Hepatitis B virus medications such as telbivudine will no longer have activity against HBV, and agents such as entecavir may be less efficacious and more prone to development of HBV resistance. As with other agents that have activity against HIV, 3TC and FTC should be used only for patients on fully suppressive ART (Benhamou *et al*., 1999).

**Entecavir**: Entecavir is a guanosine analogue that appears to be more potent than either 3TC or adefovir. Entecavir resistance requires the development of several resistance mutations, including the rtM204 mutation that confers resistance to 3TC. In the presence of 3TC resistance, entecavir usually is active but may be more vulnerable to the development of further resistance. Although entecavir initially was thought to have no anti-HIV activity, it has been demonstrated to select for the M184V mutation and should not be used in the absence of combination ART with full suppression of HIV viremia (Sherman *et al*., 2006; McMahon *et al*., 2007).

**Telbuvudine**: Telbivudine is a thymidine analogue that also selects for the HBV rtM204 mutation, which leads to 3TC cross-resistance, and should not be used after 3TC or FTC failure. Telbivudine also may have anti-HIV activity and is not recommended for use without fully suppressive ART (Benhamou, 2006).

**Adefovir**: Adefovir initially was formulated as an anti-HIV agent but was not developed for that purpose, owing to an association with renal toxicity. At lower dosages, adefovir suppresses HBV replication but is less potent than telbivudine or tenofovir. Adefovir appears to be active against 3TC-resistant HBV (Benhamou, 2006). The use of adefovir largely has been supplanted in favor of treatment with tenofovir, a related but more potent agent and one that is active against HIV. At the dosage used to treat HBV, adefovir does not appear to be active against HIV and has not been associated convincingly with the development of HIV resistance mutations such as K65R

(Sheldon *et al*., 2005). Adefovir is an option for HBV treatment in HIV-infected patients who decline or cannot take ART, but it should be used with caution.

**Tenofovir**:- Tenofovir is related to adefovir but it has more potent HBV activity and also can be used for HIV treatment. As with other agents that have activity against HIV, TDF should be used only for patients who are on fully suppressive ART. It usually is used in combination with 3TC or FTC as first-line therapy. HBV that is resistant to 3TC or adefovir can be treated effectively with TDF therapy (Nunez *et al*., 2002; Peters *et al*., 2006).

### 2.6.0 HBV Resistance

Hepatitis B virus resistance can develop after long-term exposure to oral nucleoside and nucleotide analogues, particularly in the setting of 3TC or FTC monotherapy. However, TDF can be used effectively despite the presence of 3TC or adefovir. The goal of therapy is suppression of HBV below the assay level of detection. For patients on treatment, HBV DNA should be monitored at 3-6 month intervals, along with transaminases and eAg. Virologic response is defined as a $\geq 2$ $\log_{10}$ copies/mL decrease from baseline HBV DNA after 6 months of therapy. Tenofovir-based HBV treatment will fully suppress HBV replication for the majority of patients, but that could take several years to accomplish. Development of TDF resistance despite ongoing viremia has not been documented; it is unclear whether there is a role for additional anti-HBV agents such as entecavir for patients in whom HBV replication has not been suppressed after a year or more of therapy. It is important to note that HBV and eAg may remain detectable for years despite HBV DNA suppression, and in some cases indefinitely (Nunez *et al*., 2002).

Hepatitis B virus genotyping is not recommended for HBV treatment-naive individuals, but may be considered for those who have failed to suppress HBV DNA on treatment, who have had

significant 3TC monotherapy, or who have experienced a rebound in HBV DNA levels despite continued HBV treatment (Nunez *et al*., 2002).

### 2.6.1 Monitoring on Therapy

The goal of therapy is suppression of HBV below the assay level of detection. For patients on treatment, HBV DNA should be monitored at 3-6 month intervals, along with transaminases and eAg. Virologic response is defined as a $\geq 2$ $\log_{10}$ copies/mL decrease from baseline HBV DNA after 6 months of therapy. Tenofovir-based HBV treatment will fully suppress HBV replication for the majority of patients, but that could take several years to accomplish. Development of TDF resistance despite ongoing viremia has not been documented; it is unclear whether there is a role for additional anti-HBV agents such as entecavir for patients in whom HBV replication has not been suppressed after a year or more of therapy. It is important to note that HBV and eAg may remain detectable for years despite HBV DNA suppression, and in some cases indefinitely (Nunez *et al*., 2002).

Hepatitis B virus treatment in HIV-infected individuals can be associated with hepatic decompensation and transaminase flares caused by immune responses to HBV infection; however, other causes of decompensation or increased transaminases must be considered, including drug toxicity, viral hepatitis co-infection, medication nonadherence, and development of viral resistance to current HBV treatment. Co-infected patients should be cautioned against interruption of HIV and HBV therapy, as treatment interruption can be associated with HBV viral rebound and hepatic decompensation. As a sustained loss of HBV is uncommon among HIV-co-infected patients, most of these individuals require indefinite HBV treatment with

nucleosides or nucleotides, which are given as a component of HIV treatment, if possible ((Nunez *et al*., 2002; Benhamou, 2006).

### 2.6.2 Hepatocellular Carcinoma Screening

Hepatitis B virus infection is associated with development of hepatocellular carcinoma (HCC) even in the absence of cirrhosis. The 2010 American Association for the Study of Liver Disease guidelines recommend screening for HCC every 6-12 months with alpha-fetoprotein (AFP) and with ultrasound in groups at risk of HCC, including patients with chronic HBV who are >40 years of age and those of African American ethnicity. The guidelines also recommend ongoing HCC screening for patients with evidence of cirrhosis, regardless of age or other risk factors. The benefit of surveillance in patients with chronic HBV who are <40 years of age is not well established. However, given that liver disease progresses more rapidly in HIV and HBV-co-infected patients and that HCC may be more aggressive in HIV co-infection, many experts recommend HCC surveillance for all HIV-infected patients with chronic sAg-positive HBV disease (Puoti *et al*., 2002).

### 2.6.3 Immunization against HBV in HIV infection

All HIV-infected patients who lack sAb and do not have sAg positivity or occult HBV should be vaccinated against HBV. Although transient HIV viremia has been reported after vaccination in some studies, it does not appear to be clinically relevant. Patients with HIV infection are less likely to develop protective sAb after HBV vaccination, with a response rate of 18-71%, compared with >90% for HIV-uninfected adults (Shire *et al*., 2004). Lower seroconversion rates are associated with lower CD4 cell counts, detectable HIV RNA, and HCV co-infection (Rey *et al*., 2000; Fonseca *et al*.,2005). Human immunodefficiency virus infection is also associated with lower mean HBV sAb titers and faster decline of sAb levels over time. Administration of a higher dose of HBV vaccine ("double" or "renally" dosed at 40 microgram) has been associated with a trend toward increased effectiveness compared with the standard 20 mcg dose (47% vs 34%), an effect that was strongest in patients with CD4 counts of >350 cells/μL. These data have prompted some clinics to adopt 40 mcg dosing of HBV vaccine for all HIV-infected individuals, although data to support that practice are limited (Fonseca *et al*., 2005).

Although low CD4 cell counts are associated with an impaired response to vaccination, HBV vaccination should not be deferred for patients with advanced HIV, as some individuals do develop protective antibody titers despite low CD4 cell counts. Post-vaccination sAb levels should be checked 1-2 months after the vaccination series is completed to ensure titers of ≥10 IU/L (Mast *et al*., 2006).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

The study was conducted in Kaduna town, Kaduna State, Nigeria. Kaduna state lies between latitude $09^{o}$ 02''N and $11^{o}$ 23''N and longitude $06^{o}$ 15''E and $08^{o}$ 60''E and shares boundaries with Katsina, Kano and Zamfara States to the north, Plateau and Bauchi States to the east, Nasarawa State and the Federal Capital Territory to the South, Niger State to the west. The state covers an area of approximately 45,567 $km^2$, and has an estimated population of more than six (6) million people, with three (3) senatorial zones and 23 Local Government Areas (LGAs). The three (3) senatorial zones are zone I, zone II and zone III. Zone I consists of eight (8) LGAs, zone II seven (7) LGAs and zone III eight (8) LGAs. Climatic conditions in Kaduna state is tropical with well-defined wet and dry season.

### 3.2 Study Population

The study population consisted of pregnant women attending ante-natal care in the following hospitals:

1. Barau Dikko Specialist Hospital, Ungwan-Rimi, Kaduna.
2. Yusuf Dan Tsoho General Hospital, Tudun-Wada, Kaduna.
3. 44 Nigerian Army Reference Hospital, Sokoto road, Kaduna.
4. St. Gerard Catholic Hospital, Kakuri, Kaduna.

### 3.3 Controls

Female patients visiting the hospital other than pregnant women who were non-reactive to HIV and HBV were used as control population.

### 3.4 Ethical Approval and Consent

Ethical approval to carry out the study was obtained from the ethical committee of the various hospitals, while consent was obtained from all the participants enrolled in the study.

### 3.5 Inclusion and Exclusion Criteria

The inclusion criteria included all pregnant women who gave consent to participate in the study, while the exclusion criteria was all non pregnant women and pregnant women who did not give consent to participate in the study.

### 3.6 Specimen Size

The specimen size was determined using the formula described by Mugo (2008).

$$N=\frac{Z^2PQ}{D^2}$$

Where:

N=Minimum number of specimens to be collected

Z=1.96 (standard normal distribution at 95% confidence limit)

P= Local prevalence rate of previous study on HIV and HBV co-infection among pregnant women =8.9% =0.089 (Adesina *et al.*, 2010)

Q= (1-P) =1-0.089 = 0.911

D=Tolerable error (5%)

$$N=\frac{1.96^2 \text{ x}0.089\text{x } 0.911}{0.05^2}$$

$$N=\frac{0.31147309}{0.002}$$

N=124.589

N =125

The least number of specimen to be collected for the study was calculated to be 125; hence 800 specimen were collected for the study.

### 3.7.1 Questionnaire Administration

A self designed structured questionnaire was used to obtain demographic data, clinical information and risk factors associated with HIV and HBV infection. The questionnaire was administered to all participants enrolled in the each study prior to specimen collection.

### 3.7.2 Specimen Collection and Processing

About 5ml of blood specimen was collected by a laboratory scientist by venepuncture from each of the 800 participants between August and December 2011, using a disposable sterile vacutainer needle and a 5 ml vacutainer EDTA (ethylenediamine tetra-acetic acid which is an anticoagulant) bottle. The plasma was carefully aspirated into cryovials after centrifugation for five minutes and then stored at $-4^{o}c$ until analysed.

## 3.8 Analysis of Specimen for HIV

All the 800 blood specimen collected from the pregnant women were tested for antibodies to HIV 1 and HIV 2 using the Determine test kit and the HIV positive specimen were retested using Unigold test kit.

### 3.8.1 HIV Screening using Determine

**Principle of the test:** The Determine (Abbot laboratories, U.S.A) test kit is an immunochromatographic test for the qualitative detection of antibodies to HIV 1 and HIV 2. Specimen is added to the specimen pad. As the specimen migrates through the conjugate pad, it reconstitutes and mixes with the selenium-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigen and synthetic peptide at the patient's window site. If antibody to HIV 1 and or HIV 2 are absent, the antigen selenium colloid flows pass the patients window and no red line is formed at the patient's window site. If antibody to HIV 1 and or HIV 2 are present in the specimen, the antibodies binds to the antigen selenium colloid and to the antigen at the patient's window forming a red line at the patients window site. To ensure absolute validity, a procedural control bar is incorporated in the assay device to indicate proper performance of the reagents.

**Procedure:** Prior to testing, all test kit reagents were brought to room temperature. The protective foil cover of the test kit was removed and the kit labeled with the specimen identifier. Fifty (50) microlitre of the plasma was then added to the specimen pad. The result was read after 15 minutes (a single red/pink band at the control region indicates a negative result while two bands each at the control and the patients region indicates a positive result., a single band at the patients region indicates an invalid test so should be repeated).

### Human Immune deficiency Virus retest using Uni-Gold

**Principle of the test:** The principle of the HIV test using Unigold test kit (Trinity biotech PlC, Ireland) involves immobilizing recombinant proteins that represent the immunodorminant regions of HIV 1 and HIV 2, a glycoprotein gp41, gp120 (HIV 1) and gp 36 (HIV 2) at the test

region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as the control region. During testing, 2 drops of serum, plasma or whole blood is applied to the specimen pot followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class specific to the recombinant HIV 1 and HIV 2 proteins will react with the colloidal gold linked antigen. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualized by a pink/red band at the test region of the device. A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analysed specimen; consequently no visually detectable band develops in the test region of the device. Excess conjugate forms a second pink/red band in the control region of the device. The appearance of this band indicates proper performance of the reagent in the kit.

**Procedure:** The specimens that were reactive with Determine were retested using the Uni-Gold test kit following the Federal ministry of health 2009 algorithm. The Uni-Gold test device was removed from its protective wrapper and labelled with the patients identifier. Five (5) microlitre of the plasma was placed in the specimen pot on the kit and 5microlitre of Uni-Gold buffer added. The result was read after 15 minutes (a single red/pink band at the control region indicates a negative result while two bands each at the control and the patients region indicates a positive result, a single band at the patients region indicates an invalid test so should be repeated).

### 3.8.2 CD4 count determination using Becton Dickinson Fluorosciene Activated Cell Sorter

**Principle of the test:** The BD FACS count (BD Biosciences, USA) for CD4 determination, involves fluorochrome-labeled antibodies in the reagents binding specifically to lymphocyte surface antigens when whole blood is added to the reagents, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added to the reagent tubes, the specimen is run on the instrument. During specimen acquisition, the cells come in contact with the laser light, which causes the fluorochrome-labeled cells and fluorescently dyed cells to fluoresce. This fluorescent light provides the information necessary for the instrument to identify and count the lymphocytes and CD4 lymphocytes.

**Procedure:** The BD FACS CD4 reagent tube was vortexed (mixed) and opened using a coring station. Fifty microlitre (50 µl) of the patients whole blood was added to the BD FACS CD4 reagent and the tube vortexed for 5 seconds.The tube was then incubated for 1 hour in the BD FACS workstation. Following the incubation period, 50 µl of the BD FACS fixing reagent was introduced into the tube and vortexed for 5 seconds. The tube was then placed in the specimen holder of the BD FACS CD4 Count machine and allowed to run and display the CD4 result.

### 3.8.3 HBV Screening

All the 800 blood specimen collected from the pregnant women were tested for HBsAg using the First response test kit and the HBsAg positive specimen were confifmed using Kinghawk ELISA. The HBsAg positive specimens were further tested for other HBV markers using One Step HBV multi-5 test. The tests were all carried out according to the manufacturers' instruction.

### 3.8.3.1 Antigen Detection Technique using First Response HBV Card

**Principle of the test:** The first response HBV card test (Premier medical corporation Ltd., India) is an *in vitro*, qualitative,one-step immune-chromatographic assay for the detection of HBV. The test is a two site immunometric assay in which a combination of monoclonal and polyclonal antibodies is used to selectively detect HBV in serum or plasma.

**Procedure:** All the 800 plasma specimen were tested for HBV using the First response test card. The test device was removed from the foil pouch and labeled with the specimen identifier. Twenty five (25) microlitre of plasma was placed in the specimen pot of the cassette.The reading was taken at 10 minutes (a single red/pink band at the control region indicates a negative result while two bands each at the control and the patients region indicates a positive result, a single band at the patients region indicates an invalid test so should be repeated).

### 3.8.3.2 ELISA assay using the KingHawk HBsAg kit

**Principle of the test:** The KingHawk HBsAg ELISA kit (Beijing KingHawk pharmaceutical Co., LTD, Beijing) is a double antibody sandwich ELISA designed to detect HBsAg in human serum or plasma. The anti-HBsAg is coated on the solid phase of multi-wells. Plasma samples and multiclonal anti-HBs (conjugated) are added to the coated wells. When incubated with TMB, a colored product indicates the presence of HBsAg while a non coloured is an indication of the absence of HBsAg.

**Procedure:** All specimens and reagent kits were brought to room temperature (18-25$^0$C) and mixed. The 30ml wash buffer provided was reconstituted (20x) with distilled water to 600ml. The wells were then labeled (one reagent blank well, two negative control wells, two positive control wells and specimen wells). Exactly 100µl sera and controls were dispensed into the

specimen and control wells respectively. The plate was then covered with a plate sealer and incubated for 60mins at $37^0$C without washing. Fifty microliter (50µl) of the conjugate was then added to each of the wells except the reagent blank well and the plate placed on a shaker for 5-10seconds. The plate was the covered with a plate sealer and incubated at $37^0$C for 30 minutes. The plate was then washed six (6) times with the reconstituted wash buffer each time stewing for 15-20 seconds and blotting dry. Fifty micro liters (50µl) of color reagent A and fifty micro liters (50µl) of color reagent B were then added to each well and the plate shaken, and incubated for 30minutes at $37^0$C. Fifty microliter (50µl) of the stop solution was then added to each well, the plate shaken and the bottom of the plate wiped properly. The micro plate reader (GF-M 3000) was then blanked at 450nm with the blank well and the absorbance (A) of each well read at 450nm within 30 minutes.

**Interpretation of results**:

Cutoff A value= 2 x N

Where N= Mean A value of negative control

If the test specimen A value is less than the cutoff A value, then it is considered negative, and it the test specimen A value is greater than cutoff A value then it is considered positive.

N = 0.132

Cutoff A value = 2 x 0.132

A value = 0.264

### 3.8.4 Detection of other Markers of HBV Infection

The other markers of HBV include hepatitis B surface antibody (anti-HBs), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (anti-HBe), and hepatitis B core antibody (anti-HBc).

**Principle of the test:** The One Step HBV multi-5 test (Beijing Easysweet Biomedicine Sci.Tech Co., ltd, China).The one step cassette style HBV marker test is a rapid test based on the principle of immunoassay combined with conjugated colloid gold technology.

**Procedure:** All specimens that were reactive to HBV were further analysed to detect other markers of HBV infection.The test kit was removed from the pouch and labeled with the specimen identifier. Four (4) drops of plasma was then placed in each of the specimen well. The reading was taken after 15 minutes [(coloured bands appearing in both the control and test region indicates a positive result while only one coloured band in the control region and no band in the control region indicates a positive, negative and an invalid result respectively for HBsAg, anti-HBs and HBeAg) (a dinstinct control coloured band appearing in the control region indicates a positive result, two coloured bands in the control and test regions indicates a negative result, no band in the control region means an invalid result for HBeAb and HBcAb)].

### 3.9 Data Analysis

The data obtained from the questionnaire and the results of the laboratory analysis were analyzed using SPSS 17 (statistical package for social sciences version 17) and results obtained were presented in tables, figures and graphs.

The Pearson chi-square test was used to determine the relationships between the demographic data and clinical information with HIV and HBV infection and P value < 0.05 was considered significant at 95% confidence interval.

# CHAPTER FOUR

## 4.0 RESULTS

Out of the 800 specimen tested, HIV was detected in 47 pregnant women giving a prevalence of 5.9% while HBV was detected in 31 pregnant women giving a prevalence of 3.9%. Four (4) of the pregnant women were positive for HBV and HIV giving a co-infection prevalence of 0.5% (Figure 4.1). The Venn diagram shows the total number of pregnant women that partook in the study (designated by $\varepsilon=800$). Of this number, 47 persons tested positive for HIV, 31 tested positive for HBV, while 4 persons were co-infected with both viruses and 716 persons tested negative to either viruses.

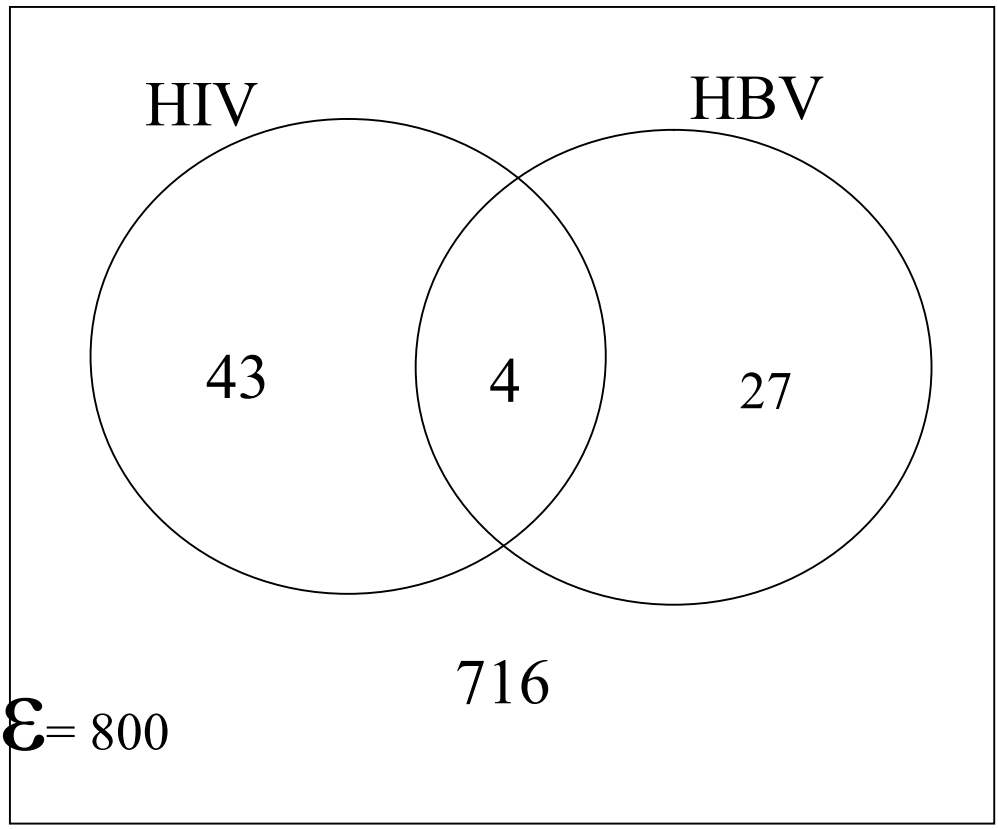



Figure 4.1: Venn diagram of the distribution of HIV and HBV and their co-infection among pregnant women attending ante-natal care in the selected hospitals in Kaduna metropolis.

The distribution of the HIV and HBV infection among pregnant women according to age group was analysed and the result shown in Table 4.1. There was no significant association (P = 0.259 for HIV and P= 0.968 for HBV) between the age of participants and the infections. For HIV infection, women in age group 36-40 years had the highest prevalence of 13% (7/54) while women in both age groups 16-20 and 26-30 years had the lowest prevalence of 4.9%. For HBV, women in the age group 21-25 years had the highest prevalence of 4.4% (10/229) while women in age group 16-20 years had the lowest prevalence of 2.9% (3/102).

The distribution of HIV and HBV infection according to gestational age was analysed and the result shown in Table 4.2. There was no significant association (P=0.773 for HIV and P=0.938 for HBV) between the gestational age and presence of the viral infections. For HIV infection, women in their first trimester had the highest prevalence of 7.9% (6/76) while those in their last trimester had the lowest prevalence of 4.9% (14/283). While for HBV, women within their second trimester had the highest prevalence 4.1% (18/440) while those within their first trimester had the lowest prevalence of 2.6% (2/76).

Table 4.1: Age distribution of HIV and HBV among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| Age group (Years) | Total No. of Specimens | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| 16 – 20 | 102 | 5 (4.9) | 97 (95.1) | 3 (2.9) | 99 (97.1) |
| 21 – 25 | 229 | 12 (5.2) | 217 (94.8) | 10 (4.4) | 219 (95.6) |
| 26 – 30 | 283 | 14 (4.9) | 269 (95.1) | 12 (4.2) | 271 (95.8) |
| 31 – 35 | 125 | 9 (7.2) | 116 (92.8) | 4 (3.2) | 121 (96.8) |
| 36 – 40 | 54 | 7 (13.0) | 47 (87.0) | 2 (3.7) | 52 (96.3) |
| >41 | 7 | 0 (0.0 | 7 (100.0) | 0 (0.0) | 7 (100.0) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(* $\chi^2 = 6.522$; df = 5; p = 0.259) (** $\chi^2 = 0.928$; df = 5; p = 0.968)

Table 4.2: Distribution of HIV and HBV by gestational age among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| Gestational Age | Total No. of Specimen | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| 1 – 3 | 76 | 6 (7.9) | 70 (92.1) | 2 (2.6) | 74 (97.4) |
| 4 – 6 | 440 | 27 (6.1) | 413 (93.9) | 18 (4.1) | 422 (95.9) |
| 7 – 9 | 283 | 14 (4.9) | 269 (95.1) | 11 (3.9) | 272 (96.1) |
| Unknown | 1 | 0 (0.) | 1 (100.0) | 0 (0.0) | 1 (96.1) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(* $\chi^2 = 1.118$; df = 3; p = 0.773) (** $\chi^2 = 0.411$; df = 3; p = 0.938)

The distribution of HIV and HBV according to educational status was analysed and the result showed that there was no significant association (P=0.332 for HIV and P=0.972 for HBV) between the women's educational status and presence of HIV and HBV. For HIV infection, women who had only quranic education had the highest prevalence (8.6%: 7/81) while those with both primary and quranic education had the lowest prevalence (2.2%: 2/93) (Table 4.3). For HBV, women with tertiary education had the highest prevalence 4.9%: 9/185) while those with both primary and quranic education had the lowest prevalence (3.2%: 3/93).

The distribution of HIV and HBV according to marital status was analysed and the result shown in Table 4.4. There was no significant association (Fisher exact test= 0.184) between marital status and HIV infection. The highest prevalence of HIV was found among widows (50%: 1/2) as against married women (5.8%: 46/798). There was no significant difference (P =0.776) in the rate of HBV infection and marital status since all the women reactive to HBV were married (3.9%: 31/800).

The distribution of HIV and HBV according to family type was analysed and the result depicted in Figure 4.2. There was a significant difference (P=0.008) in family type for HIV infection (O.R= 0.47, 95% C.I= 0.248-0.892). The highest prevalence of HIV was obtained for women who were in polygamous marriages (9.9%: 15/151) as against women in monogamous relationships (4.9%: 32/649). But there was no significant difference (P=0.944) in the rate of HBV by family type as it was observed that the prevalence among those in monogamous and those in polygamous marriages were similar (3.9% and 4.0% respectively).

Table 4.3: Distribution of HIV and HBV by educational status among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| Educational Status | Total No. of Specimen | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| Primary | 191 | 5 (2.6) | 186 (97.4) | 7 (3.7) | 184 (96.3) |
| Secondary | 339 | 23 (6.8) | 316 (93.2) | 12 (3.5) | 327 (95.9) |
| Tertiary | 185 | 12 (6.5) | 173 (93.5) | 9 (4.9) | 176 (95.1) |
| Quranic only | 81 | 7 (8.6) | 74 (91.4) | 3 (3.7) | 78 (96.3) |
| None | 4 | 0 (0.0) | 4 (100.0) | 0 (0.0) | 4 (100.0) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(* $\chi^2 = 5.740$; df = 5; p = 0.332) (** $\chi^2 = 0.873$; df = 4; p = 0.972)

Table 4.4 Distribution of HIV and HBV by marital status among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| Marital Status | Total No. of Specimen | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| Married | 798 | 46 (5.8) | 752 (94.2) | 31 (3.9) | 767 (96.1) |
| Widowed | 2 | 1 (50.0) | 1 (50.0) | 0 (0.0) | 2 (100.0) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(* $\chi^2 = 7.059$; df = 1; p = 0.008) (** $\chi^2 = 0.081$; d = 15; p = 0.776)
Fisher exact test = 0.184

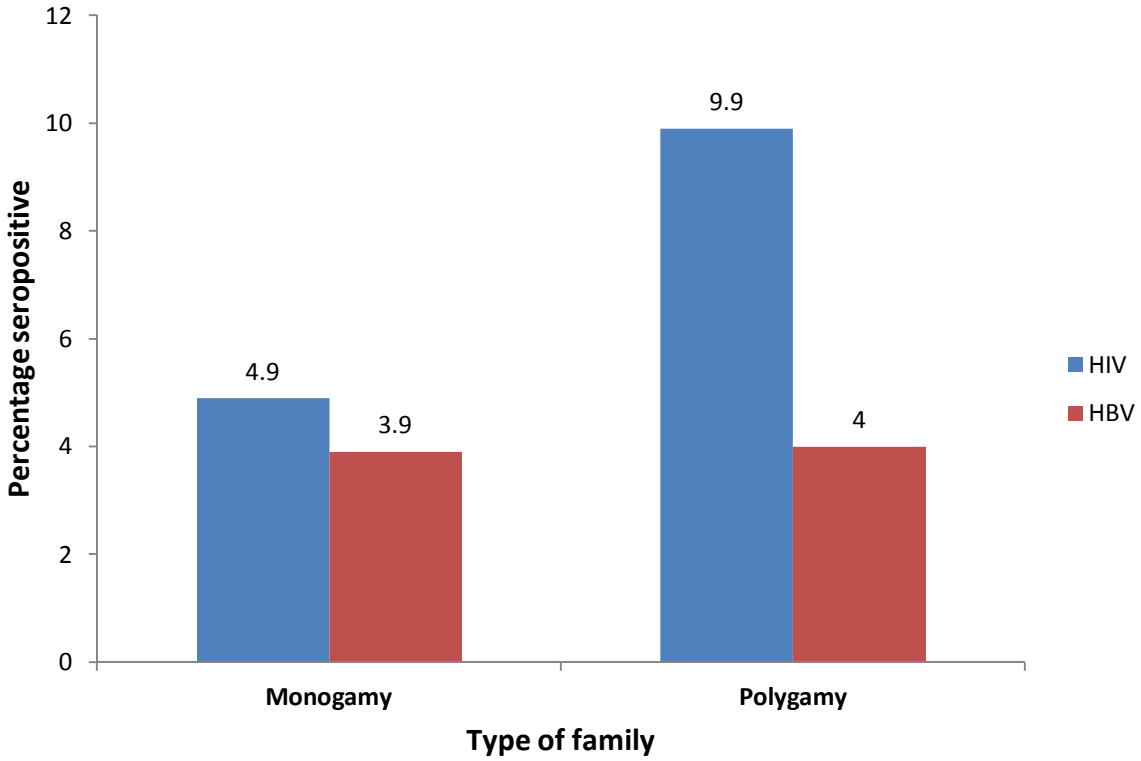



Figure 4.2: Prevalence of HIV and HBV by family type among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

With respect to women's geopolitical zones of origin there was no significant association (P = 0.654 for HIV and P= 0.250 for HBV) between the women's geopolitical zone and the viral infections (Table 4.5). However, for HIV infection, women who are from the south-south zone had the highest prevalence (10.3%:3/29) while those from the south-east region had the lowest seropositivity of (2.2%: 1/46). For HBV, women from the south-east region had the highest prevalence (8.7%: 4/46) while those from the south-west had the lowest prevalence (2.1%: 1/47).

The result of the distribution of HIV and HBV according to history of blood transfusion is shown in Figure 4.3. There was a significant difference (P < 0.001) between history of blood transfusion and HIV infection and women who had been transfused were six times likely to be infected with HIV than women who had not been transfused (O.R= 5.97; 95%C.I=2.531-14.100). The prevalence of HIV among women who have had history of blood transfusion was 24.2% (8/33) as against 5.1% (39/767) among those with no history of blood transfusion. There was no significant association (P = 0.506) between women who had been transfused and HBV. However the highest prevalence of HBV was observed in women who had history of blood transfusion (6.1%: 2/33) while those who had no history of blood transfusion had the lowest (3.8%: 29/767).

Table 4.5: Distribution of HIV and HBV according to geopolitical zone among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| Geopolitical zones | Total No. of Specimen | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| North West | 482 | 30 (6.2) | 448 (93.8) | 13 (2.7) | 465 (97.3) |
| North East | 53 | 1 (1.9) | 52 (98.1) | 3 (5.7) | 50 (94.3) |
| North Central | 143 | 9 (6.3) | 134 (93.7) | 9 (6.3) | 134 (93.7) |
| South East | 46 | 1 (2.2) | 45 (97.8) | 4 (8.7) | 42 (91.3) |
| South South | 29 | 3 (10.3) | 26 (89.7) | 1 (3.4) | 28 (96.6) |
| South West | 47 | 3 (6.4) | 44 (93.6) | 1 (2.1) | 46 (97.9) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(* $\chi^2 = 4.168$; df = 6; p = 0.654) (** $\chi^2 = 7.843$; df = 6; p = 0.250)

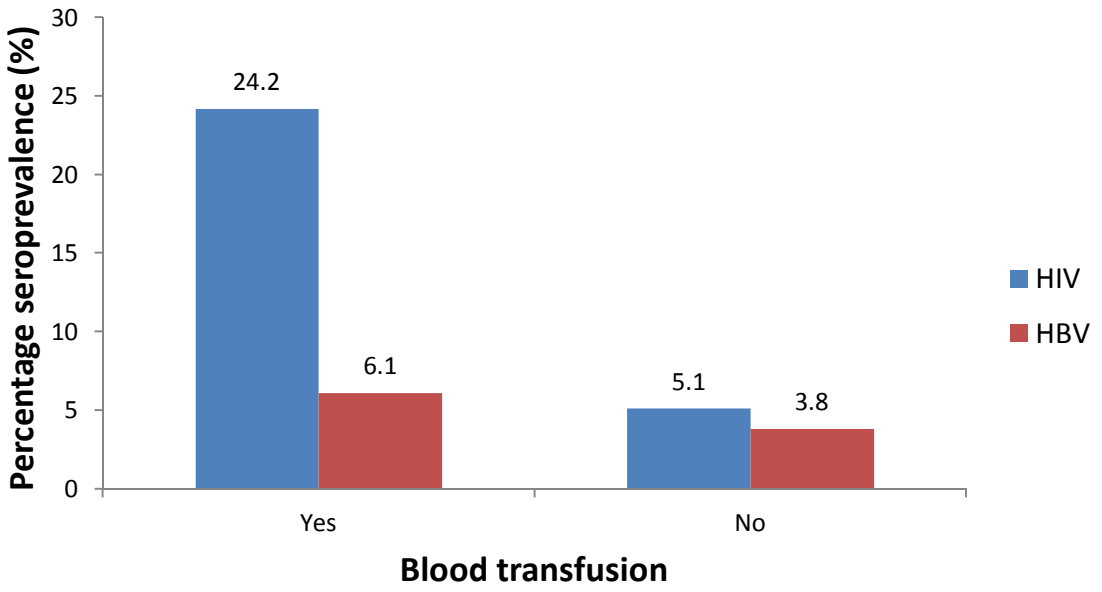



Figure 4.3: Prevalence of HIV and HBV by history of blood transfusion among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

The distribution of HIV and HBV according to presence of tribal mark and scarification was analysed and the result is shown in Figure 4.4. The result showed no significant difference (P = 0.117 for HIV and P=0.217 for HBV) between presence of tribal mark and scarification among the women and presence of the infections. For HIV infection, women who had no tribal mark and scarification had the highest prevalence of (6.7%: 38/566) while those with tribal mark and scarification had the lowest prevalence of (3.8%: 9/234) (* $\chi^2 = 2.462$; df = 1; p = 0.117). For HBV, women with no tribal mark and scarification had the highest prevalence of 4.4% (25/566) while those with tribal mark and scarification had the lowest prevalence of 2.6% (6/234) (** $\chi^2 = 1.526$; df = 1; p = 0.217).

The distribution of HIV and HBV according to history of sexually transmitted infections was analysed and the result is shown in Figure 4.5. There was no significant difference (P=0.655 for HIV and P= 0.968 for HBV) between history of sexually transmitted infections among the women and presence of the viruses. For HIV infection, women with history of sexually transmitted infections had the highest prevalence of (6.6%; 12/183) while those without history of sexually transmitted infections history had the lowest (5.7%; 35/617) (* $\chi^2 = 0.200$; df = 1; p = 0.655). For the presence of HBV, women with and those without history of sexually transmitted infections had similar prevalence (3.8% and 3.9% respectively) (** $\chi^2 = 0.002$; df = 1; p = 0.968).

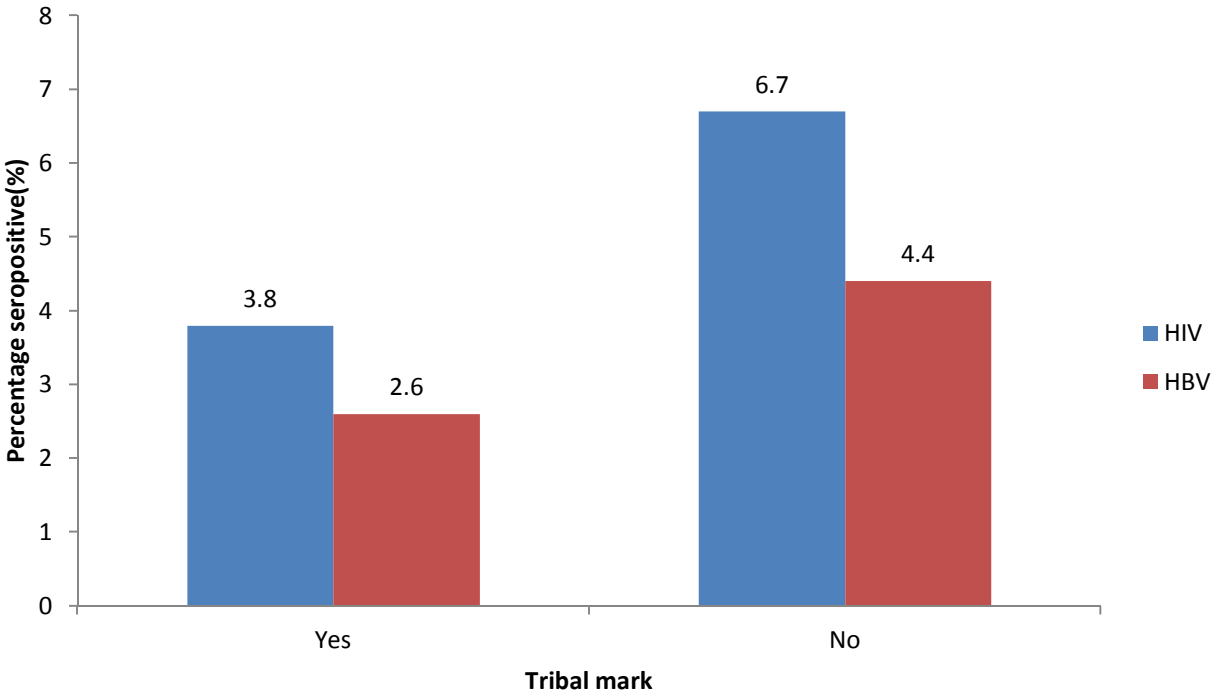



Figure 4.4: Prevalence of HIV and HBV according to presence of tribal mark and scarification among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

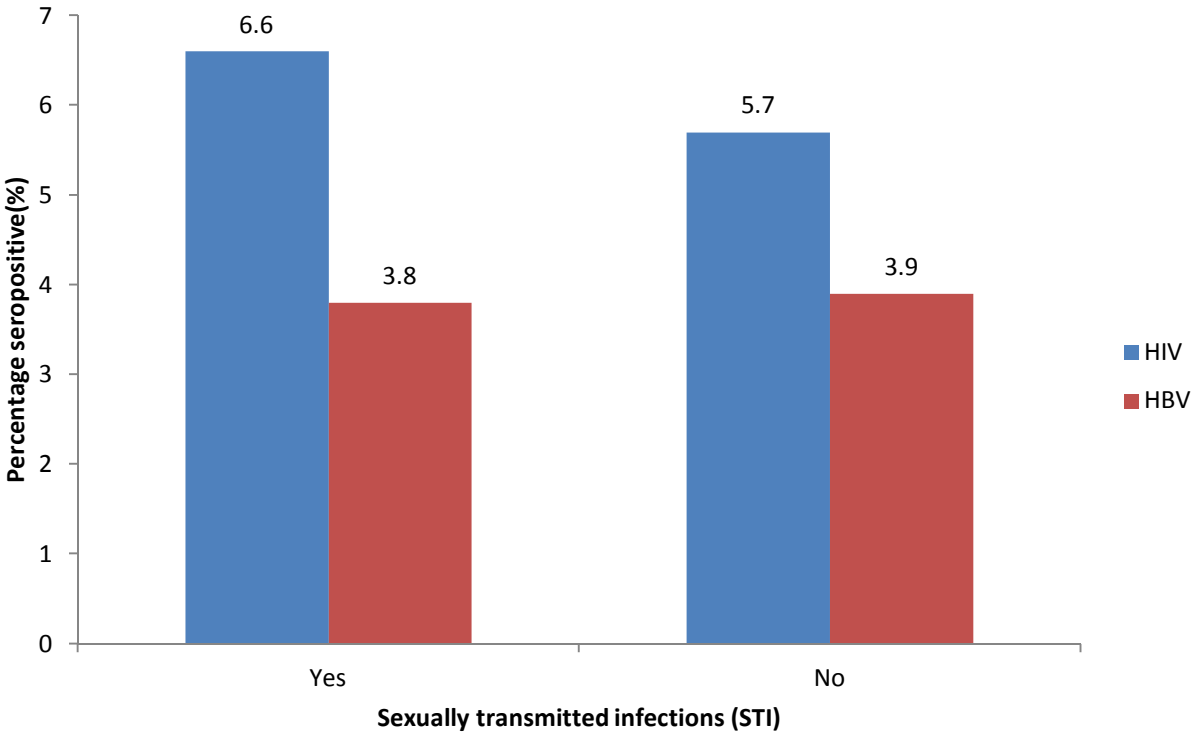



Figure 4.5: Prevalence of HIV and HBV by history of sexually transmitted infections among pregnant women attending ante-natal care in Kaduna metropolis.

The distribution of HIV and HBV according to history of injury with unsterilized objects such as nail cutter, nailfiles, cuticle removal and razor was analysed and the result is shown in Figure 4.7. There was no significant association between history of injury with unsterilized objects such as nail cutter, nailfiles, cuticle removal and razor among the women and the viral infections. For HIV infection, there was no observed statistical significant difference (*$\chi^2$ = 0.001; df = 1; P=0.982) the prevalence among those with and those without history of injury with unsterilized objects were similar (5.8%: 6/103 and 5.9%: 41/697 respectively).

For HBV, there was also no significant association (** $\chi^2$ = 0.304; df = 1; p = 0.581) however women who had history of injury with unsterilized objects had the highest prevalence (4.9%: 5/103) while those without history of injury with unsterilized objects had the lowest (3.7%: 26/697).

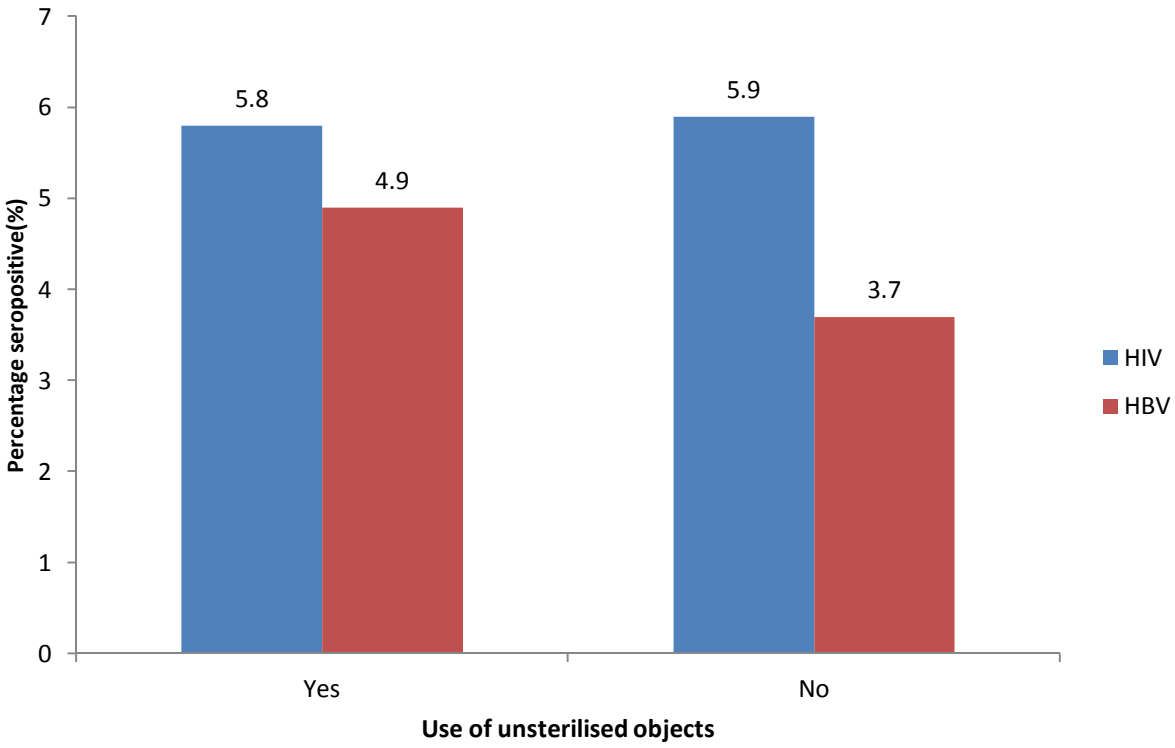



Figure 4.6: Prevalence of HIV and HBV according to history of injury with unsterilized objects among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

The distribution of HIV and HBV according to history of surgery was analysed and the result shown in Figure 4.7. There was no significant association (P=0.124 for HIV and P=0.852 for HBV) between the history of surgery and the viral infections. For HIV infection, women who had undergone surgery had the highest prevalence (10.0%: 7/70) while those who had not had the lowest prevalence (5.5%: 40/730). Although there was no significant difference, women who had undergone surgery were two times more likely to be infected with HIV than those who have not. For HBV, women with history of surgery had the highest prevalence (4.3%: 3/70) while those with no history of surgery had the lowest (3.8%: 28/730).

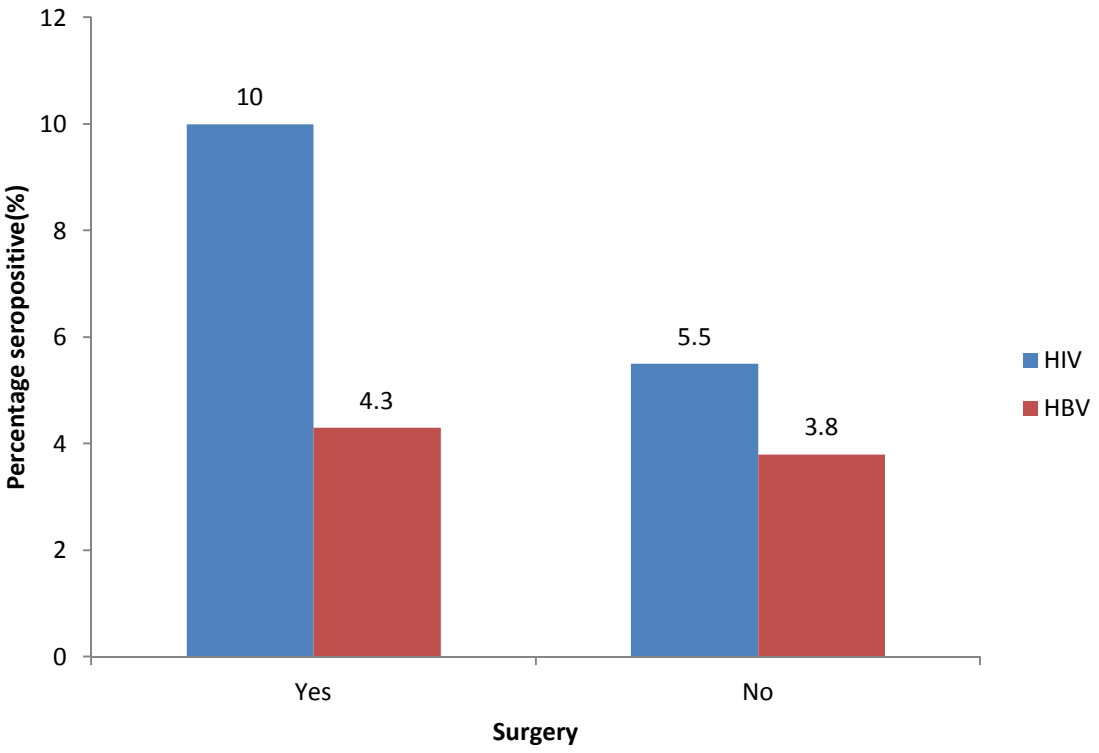



Figure 4.7: Prevalence of HIV and HBV according to history of surgery among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

The distribution of HIV and HBV according to number of previous pregnancies was analysed and the result shown in Table 4.6. There was no significant association (P=0.619 for HIV and P=0.368 for HBV) between the number of previous pregnancies and the viral infections. This study however found a higher HIV seropositivity among multigravidae (10.0%: 7/70) as compared to primigravidae (4.5%: 9/201). For HBV, the highest prevalence was found equally among those with one and those with four pregnancies (6.0%: 12/201 and 5.7%: 4/70 respectively), while the lowest was found among those with two successful previous pregnancies (1.6%: 2/127).

Table 4.6: Distribution of HIV and HBV metropolis according to number of previous pregnancies among pregnant women attending ante-natal care in some hospitals in Kaduna.

| Previous Pregnancy | Total No. of Specimen | HIV* | | HBV** | |
|---|---|---|---|---|---|
| | | No. Positive (%) | No. Negative (%) | No. Positive (%) | No. Negative (%) |
| None | 164 | 8 (4.9) | 156 (95.1) | 6 (3.7) | 158 (96.3) |
| One | 201 | 9 (4.5) | 192 (95.5) | 12 (6.0) | 189 (94.0) |
| Two | 127 | 7 (5.5) | 120 (94.5) | 2 (1.6) | 125 (98.4) |
| Three | 94 | 6 (6.4) | 88 (93.6) | 3 (3.2) | 91 (96.8) |
| Four | 70 | 7 (10.0) | 63 (90.0) | 4 (5.7) | 66 (94.3) |
| > Four | 144 | 10 (6.9) | 134 (93.1) | 4 (2.8) | 140 (94.2) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

* $\chi^2 = 3.530$; df = 5; p = 0.619 ** $\chi^2 = 5.412$; df = 5; p = 0.368

The distribution of HIV and HBV according to the number of previous sexual partners was analysed and the result shown in Table 4.7. There was no significant association (P=0.329 for HIV and P=0.263 for HBV) between the number of previous sexual partners and the viral infections. This study however found a higher HIV seropositivity among those who have had only one sexual partner (6.8%: 34/497) and the lowest seropositivity among those that have had two sexual partners (4.2%: 10/237). While for HBV, the highest prevalence was obtained among those who have had more than two sexual partners (7.6%: 5/66) while the lowest was among those that have had two sexual partners (3.4%: 8/237).

Table 4.7: Distribution of HIV and HBV among pregnant women attending ante-natal care in some hospitals in Kaduna metropolis according to number of previous sexual partners.

| **No. of Sexual partner** | **Total No. of Specimen** | **HIV*** | | **HBV**** | |
|---|---|---|---|---|---|
| | | **No. Positive (%)** | **No. Negative (%)** | **No. Positive (%)** | **No. Negative (%)** |
| One | 497 | 34 (6.8) | 463 (93.2) | 18 (3.6) | 479 (96.4) |
| Two | 237 | 10 (4.2) | 227 (95.8) | 8 (3.4) | 229 (96.6) |
| > two | 66 | 3 (4.5) | 63 (95.5) | 5 (7.6) | 61 (92.4) |
| Total | 800 | 47 (5.9) | 753 (94.1) | 31 (3.9) | 769 (96.1) |

(*$\chi^2$ = 2.224; df = 2; p = 0.329) (**$\chi^2$ = 2.671; df = 2; p = 0.263)

The distribution of other HBV serological markers in the HBsAg positive pregnant women was analysed and the result indicates that 3.2% of the women had detectable hepatitis B surface antibody (anti-HBs), 6.5% had hepatitis B envelop antigen (HBeAg), 51.6% of the women have the envelope antibody (anti-HBe), 58.1% of the women have hepatitis B core antibody (anti-HBc).

The CD4+ count result was analysed and the result showed that the HIV positive pregnant women had a mean CD4+ count of 396cells/uL of blood while a mean CD4+ count of 299cells/uL was obtained among the HIV and HBV co-infected pregnant women.

Majority of the HIV positive pregnant women (68%:32/47) had CD4+ count below 500 cells/uL of blood, while 32% (15/47) had CD4+ count above 500 cells/uL.The co-infected pregnant women all had CD4+ cell count below 600cells/uL (Figure 4.8).

The range of CD4+ cell count was determined and it was found that only 2% (1/47) of the HIV positive pregnant women had CD4+ count above 1000 cells/uL, while 98% (46/47) had CD4+ cell count below 1000 cells/uL of blood (Figure 4.9).

Table 4.8 Prevalence of some HBV markers among HBsAg positive pregnant women attending ante-natal care in some hospitals in Kaduna metropolis.

| HBV Marker | Total number of specimen = 31 | |
|---|---|---|
| | No Positive (%) | No Negative (%) |
| Anti-HBs | 1 (3.2) | 30 (96.8) |
| HBeAg | 2 (6.5) | 29 (93.5) |
| Anti-HBe | 16 (51.6) | 15 (48.4) |
| Anti-HBc | 18 (58.1) | 13 (41.9) |

KEY:

Anti-HBs - Hepatitis B surface antibody

HBeAg – Hepatitis B envelop antigen

Anti-Hbe – Hepatitis B envelop antibody

Anti-HBc – Hepatitis B core antibody

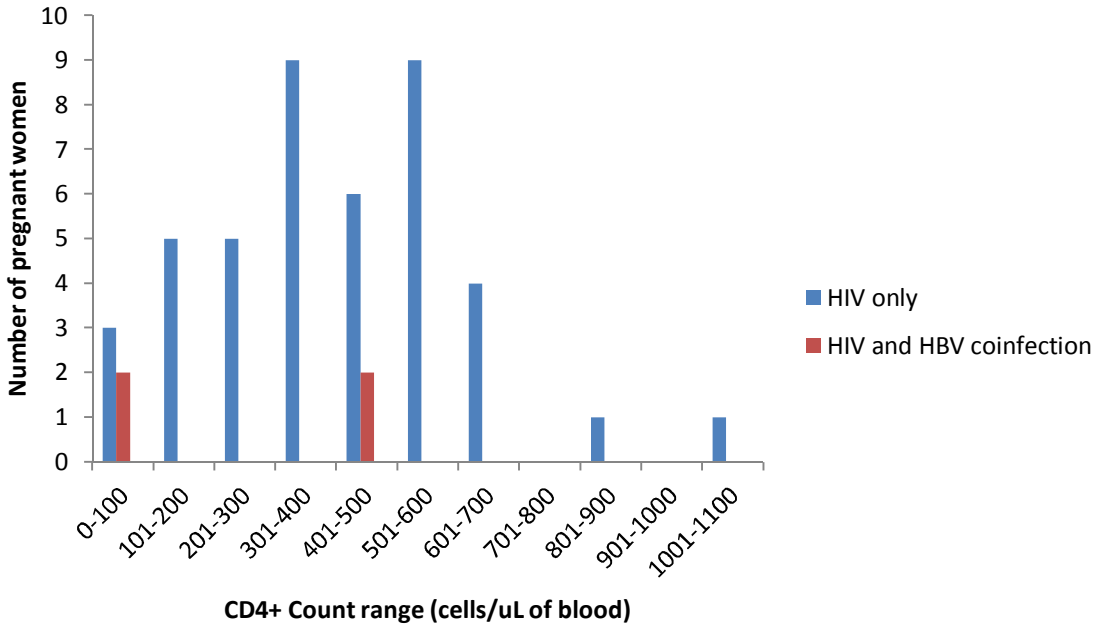



Figure 4.8: Distribution of pregnant women with HIV and its co-infection with HBV according to CD4+ count range

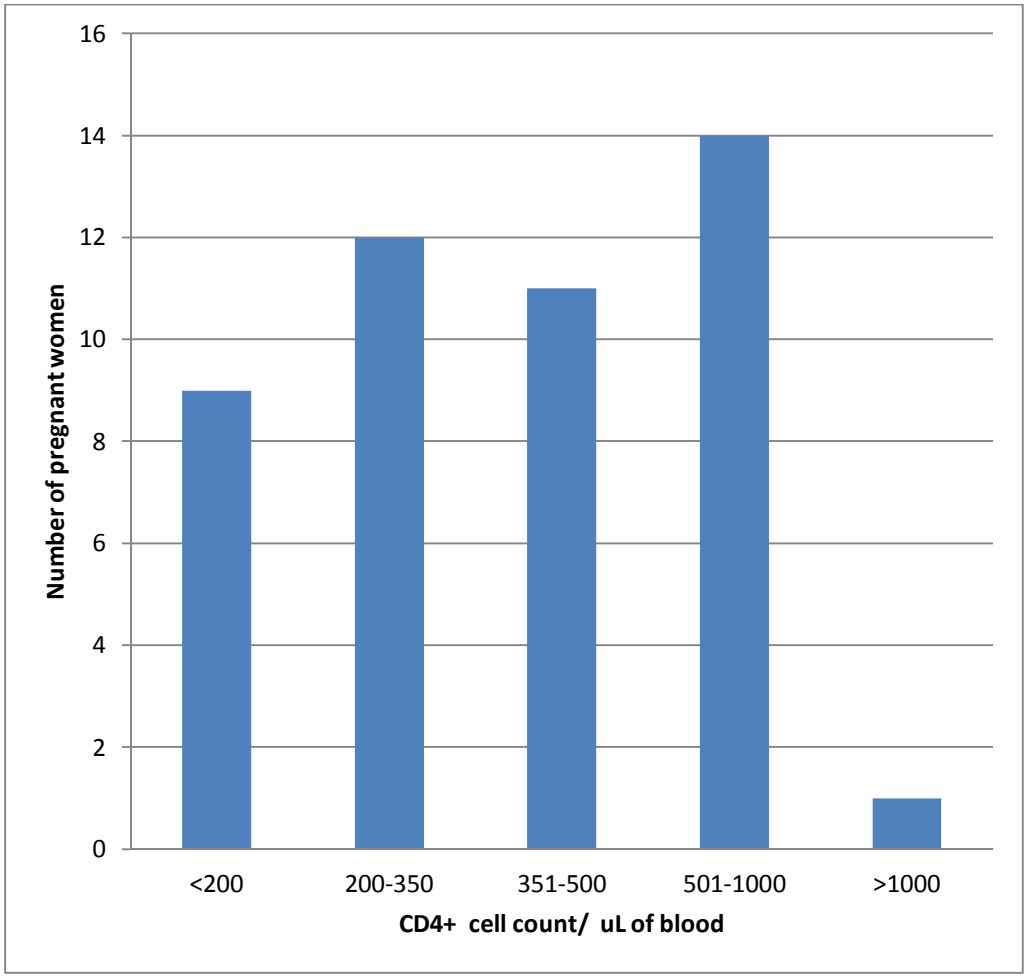



Figure 4.9: CD4+ count range amongst HIV seropositive women attending ante-natal care in Kaduna metropolis.

# CHAPTER FIVE

## 5.0 DISCUSSIONS

Results obtained from this study show that 5.9% of the pregnant women were seropositive for HIV and 3.9% seropositive for HBV, while 0.5% were co-infected with both viruses.

The prevalence obtained for HIV infection may be considered a high prevalence as it is higher than the 5% obtained for Kaduna State by the FMOH 2009 sentinel survey (FMOH, 2010c) and is similar to the 5.4% found in Abakaliki, Ebonyi State (Obi, 2005) and the 5.2% found among pregnant women attending antenatal care at Federal Medical Centre, Yola, Adamawa State (Imade *et al.*, 2004). However, the prevalence of HIV obtained in this study is lower than the 7.2% seroprevalence rate obtained in the 2008 Nigeria National HIV Seroprevalence Sentinel Survey among ante-natal clinic attendees in Bayelsa State (FMOH, 2009), and the 8.6% reported among the ante-natal attendees at Awka, Anambra State, Nigeria (Ezegbudo *et al.*, 2004).

The prevalence was also lower than the HIV seroprevalence rate of 8.7% observed and reported among ante-natal attendees in Tanzania (Swai *et al.*, 2006). On the otherhand, the prevalence rate is higher than the 3.96% obtained from a study among pregnant women attending ante-natal care at Amassoma General Hospital, Amassoma, Bayelsa State (Egesie and Mbooh, 2008) and the 3.5% found amongst the ante-natal women in the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State (Buseri *et al.*, 2008).

The implications of HIV infection in pregnancy are serious. Human immunodefficiency virus seropositive pregnant women are significantly more likely to have recurrent vulvovaginitis,

perineal tear, postpartum hemorrhage, puerperal infection, birth asphyxia and increased perinatal mortality (Obi, 2005).

The seroprevalence of 3.9% for HBV found in this study is higher than the 2.1% found among women at the point of delivery in the University of Benin Teaching Hospital, Benin City, Nigeria (Onakewhor *et al.*, 2001) and the 1.6% seroprevalence reported among women of reproductive age in Rome, Italy and the 1.9% found among pregnant women in Bali, Indonesia (Surya *et al.*, 2005). On the otherhand, this prevalence rate is slightly lower than the 4.3% prevalence found in the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State (Ojule *et al.*, 2005), and the 4.9% prevalence found among pregnant women in Europe (Landes *et al*, 2008) and it is lower than the 9.3% reported among pregnant women in Anambra State, southeastern Nigeria (Ezegbudo *et al.*, 2004). It is also lower than the 6.2% found in pregnant women in Sierra Leone by Wurie *et al* (2005) and the 5.6% reported among pregnant Sudanese women (Elsheik *et al.*, 2007). The wide variations in the seroprevalence of HBV in pregnant women from the literature may be due to geographical variation, differences in cultural practices, sexual behaviour and practices, and differences in the test methods employed to detect HBV infection (Olokoba *et al.*, 2011).

A prevalence of HIV and HBV co-infection obtained in this study was 0.5% prevalence among the pregnant women. This prevalence is low compared to the 11.9% obtained among a Nigerian cohort of HIV infected patients (Otegbayo *et al*., 2008) and the 4.47% obtained in the United States (Kim *et al*., 2008) and slightly lower than the 0.88% co-infection rate obtained by Dao *et al* (2001) in Burkina-faso and 1.13% obtained by Tounkara *et al* (2009) in Mali. This difference may be due to the difference in the study population.

Although there was no difference in the distribution of HIV infection among the various age groups the rate was highest among women in the age group 36-40 years, and lowest among women in both age groups 16-20 and 26-30 years. This suggests that women who are at the peak of their reproductive years are more prone to HIV infection as earlier postulated (Olokoba *et al*., 2011). For HBV, seropositivity was highest among the women aged between 21 and 25years, and lowest in those aged between 16 and 20 years. This finding contrast the finding of Olokoba *et al.* (2011) who found the highest prevalence in women in the age group 25-29 years and Ali and Ashraf (2007) who found the highest prevalence in women aged between 25-35 years.This result may be associated with higher sexual activities of individuals within these age groups (Olokoba *et al*., 2011). In all epidemiological studies, younger age has always been found to be the most important factor. The age of acquiring the infection is the major determinant of the incidence and prevalence rates (Olokoba *et al*., 2011).

Since HIV prevalence among young pregnant women (15-24 years) is used as a proxy for measuring rates of new infections in a population, (FMOH, 2009) the findings in this study suggest an increasing new rate of infection. This is in agreement with the findings of FMOH where the prevalence trend among pregnant women increased from 4.4% in 2005 to 4.6% in 2008 (FMOH, 2009).

In relation to gestational period, women who were in their first trimester of pregnancy had the highest seropositivity while those in their third trimester had the lowest seropositivity to HIV. In relation to HBV, women in their second trimester had the highest seropositivity and those in the first trimester had the lowest seropositivity. Vertical transmission of HBV infection is thought to be a major mode of transmission in endemic areas (Wright, 2006; Eke *et al*., 2011; Olokoba *et al*., 2011). Therefore at whatever stage a pregnant woman presents herself for ante-

natal care, her HIV and HBV status should be confirmed for effective prevention of transmission. Once exposed, the risk of developing chronic infection, defined as the presence of HBV for greater than 6 months duration, depends on the age of the individual and is highest in neonates (90%) compared to adolescents (50%) and adults (<10%) (Thio, 2003; Brooks *et al*., 2010).

This study did not find any correlation in terms of formal education and HIV seropositivity. This suggests that formal western education among women is not a main risk factor. The highest prevalence observed among those who lacked formal western education was probably because those who have formal education are knowledgeable about transmission routes for HIV and so are detered from engaging in unprotected sexual intercourse and other means of transmission, the highest seropositivity of HBV was observed in women who had tertiary education and this value is at a borderline with all the various educational status. This is in agreement with Buseri *et al.* (2010) who observed that those with low educational level are at a higher risk of the infections. Data on sexual behaviors indicate that risky behaviours are very common in Nigeria, while condom use remains low due to religious and cultural beliefs (Pauchaud *et al.*, 2002). In couples, insistence on use of condom during sex may be a sign of lack of trust towards the partner, which may generate acrimony. Unprotected sexual intercourse is the major route of human procreation among couples; and unfaithfulness on the part of any of the partners by way of being involved in unprotected extramarital relationship constitutes a risk factor for both HIV and HBV. Nonetheless, a study done in Jimma, Ethiopia among pregnant women has shown high HBV positivity rate among the illiterate (Awol and Gebre-Sellasie, 2005).

In relation to marital status, HIV seropositivity was higher among the women who were widowed as compared to the married women ($P<0.05$). The Centre for Disease Control and Prevention reported that at least 38% of women are infected through unprotected heterosexual contact with HIV-positive partner. On the other hand, marital life itself becomes a risk factor for those women who get infected by their HIV-infected spouse (Beck *et al.*, 2002). While for HBV, all the infected women were married indicating that the infection might be through unprotected heterosexual intercourse or close contact with their infected spouse as the virus can be spread through body fluids and close contact (Simpore *et al*., 2006; Willey *et al*., 2011).

In relation to family type, HIV and HBV seropositivity were highest among those in polygamy as compared to those in monogamous relationship. This may be because of the multiple sexual relationship in polygamous families as well as large population and person to person contact in polygamous homes (Otegbayo *et al*., 2008).

In relation to geopolitical zones, For HIV infection, women who were from the south-south zone had the highest prevalence while those from the south-east region had the lowest seroprevalence. For the presence of HBV, women from the south-east region had the highest seroprevalence while those from the south-west had the lowest seroprevalence. It can be seen that there are wide geographical variations in the seroprevalence rates of HIV and HBV infection amongst pregnant women within Nigeria. The variations may be a reflection of the differences in sexual practices and behaviour, awareness of HIV infection and HBV and testing, as well as sociocultural practices and accessibility to healthcare.

In relation to history of blood transfusion for both HIV infection and presence of HBV, most of the seropositive women had been transfused (24.2% and 6.1% respectively), this is against the 5.1% and 3.8% seropositive women to HIV and HBV in women who have never been

transfused. This may be due to transfusion of improperly screened blood, or seroconversion after blood transfusion. This is in contrast to Buseri *et al.* (2010) that found a high number of seropositive women among those that have not been exposed to blood transfusion. Since HBV is transmitted mainly through body fluids, unprotected sexual relationship with an infected partner or vertical transmission was a more culpable risk factor. But in agreement with Adewole *et al* (2009) who identified blood transfusion as a major risk factor for co-infection with these viruses.

In relation to tribal marks, for HIV infection, women who had no tribal mark and scarification had the highest prevalence while those with tribal mark and scarification had the lowest. For the presence of HBV, women with no tribal mark and scarification had the highest prevalence while those with tribal mark and scarification had the lowest prevalence. The result of this finding is in contrast with the finding of Adewole *et al.* (2009) who identified presence of tribal mark and scarification as a major risk factor for co-infection with both viruses.

A higher HIV seropositivity among multigravidae (10.0%) as compared to primigravidae (4.9%) (P=0.619) was observed in this study. For seropositivity to HBV, the highest prevalence was recored for those with one and those with four children (6.0% and 5.7% respectively) (P=0.368). The mean number of previous pregnancies per woman in this study was 2.17 which is approximately 2 previous pregnancies per woman, thus implying previous antenatal care visits as well as enlightenment on disease prevention and management. This higher prevalence in the multigravidae may be due to early marriage practiced previously in our environment as most women were previously not given the opportunity of formal education as marriage was chosen over education (Olokoba *et al*., 2010). This has made these women to have had longer sexually active years than the primigravidae. This result contrast the report of Buseri *et al*. 2010 which

found a significant HIV seropositivity among primigravidae (6.0%) as compared to multigravidae (3.1%) ($P<0.03$).

The distribution of HBV markers in the HBsAg reactive pregnant women indicates that 3.2% of the women had developed the anti-HBs indicating the process of immune clearance of the infection which usually occurs within 6 months of acquiring the virus as anti-HBs replaces HBV as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity (WHO, 2012), while majority (96.8%) of the women did not have the antibody indicating active infection. About 6.5% of the women had hepatitis B envelope antigen (HBeAg) which is an indication of active replicative phase of the virus and highly infectious state implying very high possibility of vertical transmission of the virus to the foetus or babies born to these women, while 93.5% of the women did not have the envelop antigen. Over half (51.6%) of the women had developed the envelop antibody (anti-HBe) which generally appears after anti-HBc, and its presence correlates to a decreased infectivity as anti-HBe replaces HBeAg in the resolution of the disease (WHO, 2012). Almost half of the women did not have the envelop antibody, while 58.1% of the women have developed hepatitis B core antibody indicating acute infection as anti-HBc is the first antibody to appear in the infection (WHO, 2012). Periodic measurements of HBV serological markers in HIV-infected patients are recommended, because new HBV infections and changes of HBV serological markers are not uncommon in patients with improved immunity after commencement of HAART (Sheng *et al*., 2007).

The mean CD4 count in HIV positive pregnant women in this study was 396 cells/ul of blood. This count is higher than the 179 cells/ul obtained in the study by Omonkhelin *et al.* (2010). However, this implies that most of the pregnant women should receive HBV vaccine in

order to prevent them from contracting the disease. This is to maximize the effectiveness of the vaccine which also should be provided early (before the CD4 cell count declines to <350 cells/μL); however, for persons with advanced immunosuppression, vaccination should also not be delayed while awaiting an increase in the CD4 cell count following antiretroviral therapy (Peters and Marston, 2012). The mean CD4 count among the HIV and HBV co-infected pregnant women was 299 cells/ul of blood. The most prevalent CD4 count in this study was 300-600 cells/ul of blood which is higher than the 100-150 cells/ul obtained by Omonkhelin *et al.* (2010).

There is also a great risk of vertical transmission of HIV and HBV during parturition and breastfeeding (Fawole *et al.*, 2002). Some women who learn of their HIV positive status during pregnancy may want to choose to terminate their pregnancy (Stevens, 2008). Infection with HIV and HBV may also adversely affect pregnancy outcome, leading to spontaneous abortion, premature delivery, intrauterine growth restrictions and low birth weight infants (Agboghoroma, 2005). The interventions in preventing the aforementioned consequences can only be applied to a woman whose HIV and HBV status is known. Therefore, determining the HIV and HBV status of pregnant women is a key factor to the success of any prevention program. The findings in this study therefore support the opinion that pregnant women should be screened for HIV and HBV infection at the first antenatal clinic visit so that adequate clinical management can be planned for them.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

This study has shown a 5.9% and 3.9% prevalence of HIV and HBV respectively among pregnant women attending ante natal care within Kaduna metropolis between August and December 2011.The prevalence of HIV and HBV co-infection among the pregnant women attending ante natal care in the selected hospitals within Kaduna metropolis was 0.5%.

There was a significant association between polygamy, history of blood transfusion and surgery and the presence of both infections.The women who had only HIV infection had higher CD4+ counts than the women who were co-infected with HBV. Over half of the women had developed anti-HBc which is an indication of acute infection .Only one woman had anti-HBs indicating immunity to HBV.

## 6.2 RECOMMENDATIONS

In view of the results obtained from this study, the following are recommended:

1. Pregnant women should be screened for both HIV and HBV during their first ante-natal visit in order to inform clinical management and prevent vertical transmission.
2. Awareness should be increased on the mode of transmission of the viral infections as well as risk involved with co-infection with both viruses as co-infection could lead to high risk of maternal mortality and morbidity.
3. Human Immunodefficiency virus positive pregnant women should be screened for hepatitis B virus in order to prevent chronic liver infections.

4. Individuals should be informed on the importance of good personal hygiene in the prevention of the infections especially hepatitis B infection that can be spread through body fluids and close personal contact.
5. Proper use of condoms should be advised in order to prevent infection and reinfection.
6. Adequate care and support programs should be organized to help persons living with the infection(s).
7. Further research work on pregnant women are required as limited data are available.
8. To reduce the morbidity and mortality associated with HIV and HBV co-infection, it is necessary to treat co-infected persons and limit liver damage from other causes (ie, drugs, alcohol, or HCV).
9. Parents should be encouraged to vaccinate their children against HBV as this could help reduce the acute and chronic infection rates as well as related complications in the vaccinated children.
10. Further research work should be done to assess the evolution of serological markers of hepatitis B virus (HBV) infection among human immunodefficiency virus infected patients receiving highly active antiretroviral therapy (HAART).

**6.3 Limitations of the Study**

1. Majority of the pregnant women who partook in the study were not able to communicate in English language, this necessitated the use of an interpreter, hence the information obtained may not be accurate.

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Appendix Ia

# MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Data
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna,
Kaduna State, Nigeria.

**MOH/ADM/744/VO.I/748** **22nd August, 2011**

MSC/SCIENCE/05751/09-010
Aba, Henrietta Oneh
Department of Microbiology
A.B.U. Zaria.

**ETHICAL APPROVAL**

I have been directed to write and inform you that approval has been granted you to commence your research on the topic "Co-infection of HIV and Hepatitis B Among Pregnant Women Attending Ante-Natal Care in Kaduna Metropolis".

2. You are to make use of vacutainers

3 Collect not more than 5mls of blood.

4. To be supervised by a Medical Lab. Scientist.

5. Submit your findings to Ministry of Health at the end of your project work.

F.A. KURAH
Secretary, Ethical Committee.

**CC:**
All Medical Directors,
Ministry of Health Facilities,
Kaduna.

Above for your information and necessary action, please.

F.A. KURAH
Secretary, Ethical Committee.

Appendix Ib

# ST. GERARD'S CATHOLIC HOSPITAL

P. M. B. 2144 Kaduna State, Nigeria. Telephone 062 - 232749
Website: www.stgerardhospital.org

Your Ref: _____ Our Ref: _____ Date March 7, 2012 20____

**The Head of Department**
**Department of Microbiology,**
**Ahmadu Bello University,**
**Zaria**

**Sir,**

**RE: ABA, HENRIETTA ONEH (ETIHICAL APPROVAL)**

The above named has been granted approval to undertake the study on the research topic " co-injection of HIV and Hepatitis B virus among pregnant women attending Ante –Natal Care in some selected Hospital within Kaduna metropolis" which this Hospital is inclusive. You are please requested to assist her where necessary.

Thank you for your usual cooperation.

Yours faithfully,

ST. GERARD'S CATH. HOSPITAL
DATE 7/3/12

**Rev. Sr. Teresa Dung**
**Hospital Administrator**

All Correspondence should be Addressed to the Administrator

Appendix Ic

44 NIGERIAN ARMY REFERENCE HOSPITAL
Sokoto Road,
P.M.B 2413,
Kaduna.
Tel: 062-831194
Email: narhk_44@yahoo.com

Ref: 44 NARHK/G1/300/50

**ABA HENRIETTA ONEH** 31 January 2012

**ETHICAL CLEARANCE**
**ABA HENRIETTA ONEH**
**MSC/SCIE/05751/2009-2010**

Reference:

A. Your Letter dated 8 November 2011.

1. The above named has been granted clearance as requested vide Reference A to undertake the study as "**CO-INFECTION OF HIV AND HEPATITIS B VIRUS AMONG PREGNANT WOMEN ATTENDING ANTE-NATAL CARE IN SOME SELECTED HOSPITALS WITHIN KADUNA METROPOLIS''** which this hospital is inclusive. In view of the above you are please requested to assist her where necessary.

2. Thank you for your usual cooperation.

**IJ MAMZA**
Captain
for Director

**Appendic II**

**DEPARTMENT OF MICROBIOLOGY**
**AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.**

INFORMED CONSENT FORM (ICF)

Serial No.: ……. Hospital No.: …… Age: …….. Phone No.: ……..……

This Informed Consent Form is basically for pregnant women attending ante-natal care in some selected Hospitals in Kaduna State.We are inviting you to participate in this research work titled "Co-infection of HIV and HBV among pregnant women attending ante-natal care in Kaduna metropolis, Kaduna State, Nigeria. The research will involve the collection of blood samples of participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this hospital.

CERTIFICATE OF CONSENT

I ……..of …….. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that blood sample will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……..…
Signature/right thumb print of participant ……. Date …….

**Statement by Witness:**

I have witnessed the accurate reading of the consent form and detail explanati the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness……
Signature of witness…….…. Date ……..

**Statement by the Researcher/Person Taking Consent**

I confirm that sufficient information, including risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher …….
Signature ……... Date. ……

Appendix III

**QUESTIONNAIRE**

RESEARCH TOPIC: CO-INFECTION OF HIV AND HBV AMONG PREGNANT WOMEN ATTENDING ANTE-NATAL CARE IN KADUNA METROPOLIS.

SECTION A: BIODATA

1.Reference No:……..

2.Age: 16-20( ) 21-25( ) 26-30( ) 31-35( ) 36-40( ) >41( )

3. Gestational age ……..……months

4.Educational level: Tertiary () Secondary ( ) Primary ( )Quranic ( )none ( )

5.Marital status: Single ( ) Married ( ) Widowed ( ) Separated ( ) Divorced ( )

6.Type of family:Monogamous ( ) Polygamous ( )

7. Occupation ……

8. Husbands occupation ……

9. State of origin: ……

SECTION B: CLINICAL DATA

1. For how long have you been hypertensive?

Never( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs ( ) 16-20yrs ( ) >20yrs( )

2. For how long have you been diabetic?

Never( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs ( ) 16-20yrs( ) >20yrs( )

3. Presenting symptom of ailment ……

Fatigue Yes ( ) No ( )

Headache Yes ( ) No ( )

DizzinessYes ( ) No ( )

Stomach ache Yes ( ) No ( )

Nausea Yes ( ) No ( )

Others……

SECTION C: RISK FACTORS

1. How many times have you received blood transfusion?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

2. How many times have you received tribal marks or tattoo? Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

3.How many sexual partners have you had in the past years?

One ( ) Two ( ) >Two ( )

4. How many times have you had a sexually transmitted infection?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

5. How many times have you been exposed to intravenous drug abuse?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

6. Number of previous pregnancies: None( ) One ( ) Two ( ) Three( ) Four ( )> Four( )

7. Do you share unsterilized sharp objects such as razor, cuticle removal etc?

Yes ( ) No ( )

8. How many times have you undergone surgery in the past years?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

9. History of smoking

None( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs( ) 16-20yrs( )>20yrs( )

10. History of alcohol consumption

None( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs( ) 16-20yrs( ) >20yrs( )

11. How many times have you received ear or body piercing?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

Thank you.

Appendix IV

| S/N | REF NO | CD4 | S/N | REF NO | CD4 |
|---|---|---|---|---|---|
| 1 | BD 87 | 200 | 22 | SG 082 | 218 |
| 2 | BD 95 | 156 | 23 | SG 085 | 348 |
| 3 | BD 103 | 122 | 24 | SG 089 | 376 |
| 4 | BD 104 | 99 | 25 | NA 003 | 535 |
| 5 | BD 131 | 54* | 26 | NA 076 | 600 |
| 6 | BD 142 | 445 | 27 | NA 109 | 338 |
| 7 | BD 168 | 175 | 28 | NA 117 | 212 |
| 8 | BD 184 | 540 | 29 | NA 157 | 300 |
| 9 | BD 186 | 375 | 30 | NA 168 | 452 |
| 10 | BD 188 | 823 | 31 | NA 172 | 600 |
| 11 | BD 199 | 535* | 32 | NA 176 | 338 |
| 12 | BD 206 | 1082 | 33 | YD 006 | 511 |
| 13 | BD 222 | 241 | 34 | YD 017 | 459 |
| 14 | BD 224 | 385 | 35 | YD 018 | 8* |
| 15 | SG 003 | 600* | 36 | YD 032 | 657 |
| 16 | SG 012 | 600 | 37 | YD 034 | 58 |
| 17 | SG 014 | 338 | 38 | YD 085 | 451 |
| 18 | SG 017 | 699 | 39 | YD 106 | 437 |
| 19 | SG 018 | 176 | 40 | YD 107 | 29 |
| 20 | SG 023 | 404 | 41 | YD 128 | 628 |
| 21 | SG 076 | 293 | 42 | YD 161 | 431 |
| 43 | YD 182 | 638 | 46 | YD 227 | 338 |
| 44 | YD 185 | 476 | 47 | YD 232 | 312 |
| 45 | YD219 | 521 | | | |

KEY:

BD- Barau Dikko specialist hospital

SG- St. Gerard catholic hospital

NA- 44 Nigerian Army reference hospital

YD- Yusuf Dan Tsoho memorial hospital

*- HIV Coinfection with HBV

Appendix V

# Analysis Report

Plate No.:1
1st Item:HBsAg
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength=450
Blank ABS:0.034

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hole | B | S1 | S2 | S3 | S4 | S5 |
| OD | 0.034 | 1.873 | 0.132 | 0.161 | 0.158 | 0.172 |
| Quant | | 1.873 | 0.132 | 0.161 | 0.158 | 0.172 |
| Quali | | | | | | |
| Hole | S12 | S13 | S14 | S15 | S16 | S17 |
| OD | 0.142 | 0.120 | 0.141 | 0.127 | 1.826 | 1.865 |
| Quant | 0.142 | 0.120 | 0.141 | 0.127 | 1.826 | 1.865 |
| Quali | | | | | | |
| Hole | S24 | S25 | S26 | S27 | S28 | S29 |
| OD | 0.179 | 0.206 | 0.170 | 0.228 | 2.037 | 2.002 |
| Quant | 0.179 | 0.206 | 0.170 | 0.228 | 2.037 | 2.002 |
| Quali | | | | | | |
| Hole | S36 | S37 | S38 | S39 | S40 | S41 |
| OD | 1.775 | 1.945 | 2.041 | 2.066 | 2.081 | 1.970 |
| Quant | 1.775 | 1.945 | 2.041 | 2.066 | 2.081 | 1.970 |
| Quali | | | | | | |
| Hole | S48 | S49 | S50 | S51 | S52 | S53 |
| OD | 0.235 | 1.908 | 0.394 | 0.152 | 0.217 | 0.149 |
| Quant | 0.235 | 1.908 | 0.394 | 0.152 | 0.217 | 0.149 |
| Quali | | | | | | |
| Hole | S60 | S61 | S62 | S63 | S64 | S65 |
| OD | 0.187 | 0.142 | 1.846 | 0.126 | 0.136 | 0.136 |
| Quant | 0.187 | 0.142 | 1.846 | 0.126 | 0.136 | 0.136 |
| Quali | | | | | | |
| Hole | S72 | S73 | S74 | S75 | S76 | S77 |
| OD | 0.164 | 0.156 | 0.186 | 0.173 | 0.167 | 0.159 |
| Quant | 0.164 | 0.156 | 0.186 | 0.173 | 0.167 | 0.159 |
| Quali | | | | | | |
| Hole | S84 | S85 | S86 | S87 | S88 | S89 |
| OD | 0.124 | 0.118 | 0.218 | 0.122 | 0.092 | 0.083 |
| Quant | 0.124 | 0.118 | 0.218 | 0.122 | 0.092 | 0.083 |
| Quali | | | | | | |

| | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Hole | S6 | S7 | S8 | S9 | S10 | S11 |
| OD | 1.953 | 0.178 | 0.157 | 0.164 | 0.164 | 0.556 |
| Quant | 1.953 | 0.178 | 0.157 | 0.164 | 0.164 | 0.556 |
| Quali | | | | | | |
| Hole | S18 | S19 | S20 | S21 | S22 | S23 |
| OD | 0.160 | 0.156 | 0.150 | 1.119 | 1.204 | 1.580 |
| Quant | 0.160 | 0.156 | 0.150 | 1.119 | 1.204 | 1.580 |
| Quali | | | | | | |
| Hole | S30 | S31 | S32 | S33 | S34 | S35 |
| OD | 1.993 | 2.186 | 2.219 | 2.152 | 2.150 | 2.318 |
| Quant | 1.993 | 2.186 | 2.219 | 2.152 | 2.150 | 2.318 |
| Quali | | | | | | |
| Hole | S42 | S43 | S44 | S45 | S46 | S47 |
| OD | 2.006 | 2.012 | 0.173 | 1.883 | 0.164 | 0.207 |
| Quant | 2.006 | 2.012 | 0.173 | 1.883 | 0.164 | 0.207 |
| Quali | | | | | | |
| Hole | S54 | S55 | S56 | S57 | S58 | S59 |
| OD | 0.382 | 0.148 | 0.287 | 0.141 | 0.200 | 0.190 |
| Quant | 0.382 | 0.148 | 0.287 | 0.141 | 0.200 | 0.190 |
| Quali | | | | | | |
| Hole | S66 | S67 | S68 | S69 | S70 | S71 |
| OD | 0.161 | 0.131 | 0.294 | 0.122 | 0.148 | 0.164 |
| Quant | 0.161 | 0.131 | 0.294 | 0.122 | 0.148 | 0.164 |
| Quali | | | | | | |
| Hole | S78 | S79 | S80 | S81 | S82 | S83 |
| OD | 0.186 | 0.161 | 0.202 | 0.158 | 0.175 | 0.171 |
| Quant | 0.186 | 0.161 | 0.202 | 0.158 | 0.175 | 0.171 |
| Quali | | | | | | |
| Hole | S90 | S91 | S92 | S93 | S94 | S95 |
| OD | 0.104 | 0.094 | 0.107 | 0.099 | 0.109 | 0.137 |
| Quant | 0.104 | 0.094 | 0.107 | 0.099 | 0.109 | 0.137 |
| Quali | | | | | | |

Test date:2011-12-14 Test time:21:17:35
Print date:2011-12-14 Print time:21:28:18
User: Verifier: