SEROPREVALENCE OF HEPATITIS B VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AMONG STUDENTS IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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

OCTOBER, 2014

DEDICATION

This thesis is dedicated to Almighty God and my dear mother Mrs. Rebecca Isa.

DECLARATION

I declare that the work in this thesis entitled: "SEROPREVALENCE OF HEPATITIS B VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AMONG STUDENTS IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA" has been performed by me in the Department of Biological Sciences under the supervision of Prof. S.A. Abdullahi and Dr. (Mrs) M. Aminu-Mukhtar. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Ibrahim ISA _____ _____

Signature Date

CERTIFICATION

This thesis entitled "**SEROPREVALENCE OF HEPATITIS B VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS AMONG STUDENTS OF AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**" by Ibrahim ISA meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Hepatitis B virus (HBV) and Human Immunodeficiency Virus (HIV) are endemic in sub-Saharan Africa. Evidence on HBV co-infection rates with HIV infection among individuals remains conflicting. The study was conducted to determine the seroprevalence of HBV and HIV infections and the possible potential risk factors among students of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The cross-sectional study was conducted between April and August, 2013. Structured questionnaires were administered to obtain socio demographic data and possible risk factors that might be associated with the viral infections. Blood samples were collected at the University Health Services (UHS) from 600 consenting consecutive students aged between 16 and 40 years old. The sera were screened for HBsAg using device kit and anti-HBs, HBeAg, anti-HBe and anti-HBc using one-step cassette style diagnostic kits. Human Immunodeficiency Virus infection was screened for using determine^{TM} HIV-1/2 kit. Reactive sera for HBsAg were further confirmed using ELISA kits. Of the 600 students tested for HBsAg, 9.2% (55/600) tested positive among which, none had detectable anti-HBs antibodies, indicating recent infection. About 7.3%, 36.4% and 94.5% were positive for HBeAg, anti-HBe and anti-HBc respectively. The seroprevalence of HIV infection was 2.8% (17/600). One (0.2%) of the students was infected with both HBV and HIV. There was a significant association between age group, gender, family history of the students and HBV infection (P=0.016, 0.049 and 0.000 respectively). While for HIV, only menial jobs (p = 0.001) was significantly associated with the infection. Other risk factors studied were not significantly associated with the viral infections. The seroprevalence of 9.2% for HBsAg obtained in this study indicates high endemicity according to WHO classification. Four of the students were highly infectious. HIV and co-infection were observed to be very low. The study indicates that close contact among family members and economic disadvantages of some of the students might be a predisposing factor to the infections.More than half of the students were ignorant about HBV.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency syndrome
ART	Anti Retro Viral Therapy
Anti-HBc	Antibodies to the HBV core antigen
Anti-HBe	Antibodies to the HBV e antigen
Anti-HBs	Antibodies to the HBV surface antigen
cccDNA	Covalently closed circular DNA
cDNA	Complimentary DNA
cm	Centimeter
°C	Degrees celsius
DNA	Deoxribonucleic acid
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
HAV	Hepatitis A virus
HBcAg	Hepatitis B virus core antigen
HBeAg	Hepatitis B virus e antigen
HBsAg	Hepatitis B virus Surface antigen
HBV	Hepatitis B virus
HBx	Hepatitis B virus x protein
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis delta virus
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IgM	Immunoglobulin M
IgG	Immunoglobulin G
Kb	kilobase
µg	Microgram
UHS	University Health Services
mg	Milligram
min	Minute

µl	Microliter
ml	Milliliter
µm	Micron
µM	Micromolar
mRNA	Messenger RNA
NIH	National Institute of Health
Nm	Nanometer
NACP	National AIDS Control Program
nt	Nucleotide
OBI	Occult hepatitis B virus infection
OD	Optical density
OHB	Occult hepatitis B
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
SS	single Stranded
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

Hepatitis B Virus (HBV) is a DNA virus belonging to the family *Hepadnaviridae*with a very high transmissibility (Dienstag and Isselbacher, 2005). This virus has been detected in peripheral mononuclear cells, tissues of pancreas, spleen, kidney, skin and fluids like saliva, semen, sweat, breast milk, tears, urine and vaginal secretion (Elgouhari *et al*., 2008) and faeces (Willey *et al.*, 2011). It establishes a chronic infection especially in those infected as infants. There are high risk groups for HBV infection; these include parenteral drug users, institutionalized persons, health care personnel, organ transplant patients, haemodialysis patients and staff, highly promiscuous persons and infants born to infected mothers.

Hepatitis B virus has been described as a major public health problem or risk, occurring endemically, in all areas of the world. Approximately 1million persons die each year (2.7% of all death) from viral hepatitis related causes (WHO, 2011). An estimated 57% of cases of Liver cirrhosis and 78% of cases of 1% liver cancer results from HBV or HCV infection (WHO, 2011).However, 80% of countries identified hepatitis as an urgent public health problem(WHO, 2011).

Ithas been estimated that about 2 billion people have been infected with hepatitis B virus and 350 million have chronic lifelong infection (Ballah*et al.,* 2012). About 50million people are chronic carriers in Africa with the carrier rate ranging from 9 to 20% in sub-Saharan Africa Ballah *et.al.*, 2012.Studies done in Nigeria showed HBV carriage rate in the range of 9 to 39% (Emechebe *et. al.,* 2009).

Hepatitis B virus and HIV infections continue to be a substantial and devastating health problem, with new cases still being reported annually (Seyed-Moayed, 2006).

Studies have shown that the prevalence of HBV infection in antenatal population is a reliable indicator of HBV prevalence rate in the general population (Ahizechukwu *et. al*., 2011). Screening students for HBsAg can also give a reliable prevalence of the disease in a population, since they fall within the sexually active group and are exposed to the risk factors.

However, in Nigeria, screening of tertiary institutions students for HBV and HIV co-infection is not a routine practice. Also, routine vaccination of students with HBV vaccines is not widely available especially in low resource settings as Nigeria. Even though Nigeria approved the inclusion of hepatitis B vaccine in its National Program on Immunization (NPI) in 1995, the vaccine only became widely available in 2004 (Sadoh, and Eregie, 2008). Despite the availability of free vaccine through the NPI, immunization coverage for Hepatitis B is still not optimal in Nigeria (Sadoh, and Eregie, 2008).

A study in Nigeria showed that the Hepatitis B vaccine coverage rate is 36.2% in those that received full coverage of three doses, while 64.5% had received at least one dose of HBV vaccine (Ogoina et. al., 2014). The consequences of the problems of low pick up rate of HBV infection due to poor screening and the low vaccination rate are that vertical transmission of the virus has become the major route of transmission of the virus in Nigeria in addition to heterosexualrelationship (Ahizechukwu *et. al.,* 2011).

Human immunodeficiency virus (HIV), is a member of the *Lentivirus* genus and family *Retroviridae* that was unknown until first discovered in 1981 among homosexual men in the United States (UNAIDS, 2004). It is the pathogen responsible for the Acquired

Immunodeficiency Syndrome (AIDS) which is marked by a steady decline in the capacity of the body to fight infections and has been adjudged the most significant emerging infectious disease of the last century and continues to create health, social and developmental problems particularly in Africa (Aljabri and Saraswathi, 2012). An estimated 35million people are living with HIV infection worldwide(WHO, 2014).

Currently, two genetically and immunologically distinct human immunodeficiency viruses are known; HIV-1 and HIV -2 (UNAIDS 2004). HIV-1 is more virulent and has the widest distribution in Europe and America, while HIV-2 has the widest distribution in West Africa (Aljabri and Saraswathi, 2012).

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. Persons with HIV and chronic HBV coinfection have higher levels of HBV DNA and lower rates of clearance of the hepatitis B e antigen (HBeAg).Serum transaminase levels may be lower in HIV/HBV-coinfected patients than in HBV-monoinfected patients, but normal transaminase levels should not be interpreted to mean that there is no underlying hepatic fibrosis(Luetkemeyer 2010).

HIV increases the risk of cirrhosis and end-stage liver disease in HBV coinfection. 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/HBV-coinfected patients than in HIV-monoinfected patients (14% vs 6%). Lastly, HIV coinfection is associated with more frequent flares of hepatic transaminases, which can occur with immune reconstitution inflammatory syndrome (IRIS) owing to ART,

interruption of HIV/HBV treatment, or the development of resistance to HIV/HBV treatment; they also can occur spontaneously (Luetkemeyer 2010).

In Nigeria, a country where HBV and HIV infection prevalences are high, HBV co-infection occurs in 10%–70% of HIV-infected individuals (Iwalokun *et al.,* 2006; Nwokedi *et. al.*, 2006).Although, very few co-infection studies have been carried out in Africa but since sub-Saharan Africa is a region that accounts for two-thirds of the global HIV/AIDS burden, high HIV-HBV co-infection is expected (Hajizadeh 2014).

Furthermore, since HBV and HIV infections have serious sequelae, there is a continuing need to examine HIV or HBV epidemiology, especially in low resource settings such as Nigeria.

1.1 Statement of Research Problem

Hepatitis B Virus is 50-100 times more infectious than HIV and 10 times more infectious than hepatitis C virus and because it replicates profusely and produces high titer in the blood (10^8-10^{10} virions mL^{-1}) any parenteral or mucosal exposure to infected blood poses a high risk of HBV acquisition (Pennap *et. al.*, 2011).

Hepatitis B Virus is a serious public Health problem. Nigeria is one of the numerous countries in which HBV infection is highly endemic (Aminu *et al.*, 2013; Mbaawuaga *et al*., 2008). It is estimated that about 12% of Nigerians are chronic carriers (Mbamara, 2010). Further, studies conducted in Nigeria have shown varying seroprevalence of HBV among various populations ranging from 12.5% among tertiary institution students (Aminu *et. al.*, 2013).

Furthermore, seroprevalence studies on co-infection of HBV and HIV have been conducted in Kaduna State. Co-infection rate of0.5%had been reported amongst pregnant women (Aba *et. al.,* 2012). Although, literature on HBV infection in Nigeria is growing, yet, there is paucity of information among the youths who are known to be a group that is a high risk because of their sexualactivity.They also form the bulk of the group that is usually required when there is need for blood donation, which may lead to the spread of the viral infection, since most of the hospital lack facilities to properly screened blood.

Hepatitis B Virus infection is widely referred to as a silent killer because many carriers do not realize they are carrying the virus (Pennap *et. al.,* 2011) and hence fail to seek appropriate medical attention, therefore posed a high risk to its victim.

1.2 Justification

Hepatitis B Virus or HIV in other populations such as pregnant women, blood donors is well documented in the literatures (Jatau and Yabaya, 2009; Abubakar, 2012; Aba*et. al.,* 2012; Otori *et al.,* 2012) but there is dearth of published information on HBV and HIV especially among students of tertiary institutions.

Zaria is a mobile city, where people move in and out and interact with one another. Therefore, this study will provide the information and data needed for initiation of programmes designed to prevent and control HIV and HBV infections and to aid the students of ABU Zaria in establishing trust through the knowledge of the present prevalence rate of the infection among the students. Also, to create awareness about the aetiology of infections, mode of transmission and their public health significance.

This study generated basic information for further research studies that might improve the prevention measures of these viruses and came out with recommendations for possible measures to be taking.

There is need to carryout studies in order to alert the students on the effect of poor hygiene and unnecessary body contact as could be a possible means of acquiring HBV infection.

I.3 Aim

The aim of this study is to determine the seroprevalence of hepatitis B and Human Immunodeficiency viral infections among students of Ahmadu Bello University, Zaria-Nigeria.

1.4 Objectives

The objectives of this study were to:

1. Determine the seroprevalence and level of infection of HBV among students of Ahmadu Bello University, Zaria
2. Determine the seroprevalence of HIV infection among students of ABU, Zaria
3. Determine the HBV and HIV co-infection among students of ABU Zaria
4. Determine the potentials risk factors that might be associated with infection by these viruses.

1.5 Null Hypotheses

1. There are no cases of HBV among students of Ahmadu Bello University, Zaria.
2. There are no cases of HIV infection among students of ABU, Zaria
3. There are no known risk factors associated with the infection two viruses in ABU, Zaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hepatitis B Virus Taxonomy

Hepatitis B virus (HBV) is a member of the *Hepadnavirus* family(Schaefer, 2007; Brooks *et al.* 2010). Hepatitis B virus is a *hepadnavirus* (*hepa* from *hepatotrophic* and *dna* because it is a DNA virus). It has a circular genome composed of double-strandedDNA. Brooks *et. al.,* 2010). Hepadnaviruses can be found in both mammals (orthohepadnaviruses) and birds (avihepadnaviruses). The genetic variability of HBV is very high. There are nine genotypes of HBV and three clades of HBV isolates from apes that appear to be additional genotypes of the virus (Schaefer,2007). Most genotypes are now divided into subgenotypes with distinct virological and epidemiological properties. In addition, recombination among HBV genotypes increases the variability of HBV (Schaefer, 2007).

Hepatitis B virus is a partially double stranded virus that uses reverse transcriptase in its replication cycle. The virus replicates 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).

2.2 History of Hepatitis B Virus

Hepatitis B (formerly called serum hepatitis) has been recognized as separate entities since the early 1940s and can be diagnosed with specific serologic tests. Epidemic jaundice was described by Hippocrates in the 5th century BCE. The first recorded cases of "serum hepatitis," or hepatitis B, are thought to be those that followed the administration of smallpox vaccine containing human lymph to shipyard workers in Germany in l883. In the early and middle parts of the 20th century, serum hepatitis was repeatedly observed following the use of contaminated needles and syringes (CDC, 2012). The role of blood as a vehicle for virus transmission was further emphasized in 1943, when Beeson described jaundice that had occurred in seven recipients of blood transfusions. Australia antigen, later called hepatitis B surface antigen (HBsAg), was first described in 1965, and the Dane particle (complete hepatitis B virion) was identified in 1970. Identification of serologic markers for HBV infection followed, which helped clarify the natural history of the disease. Ultimately, HBsAg was prepared in quantity and now comprises the immunogen in highly effective vaccines for prevention of HBV infection (CDC, 2012).

World Hepatitis Day, observed July 28, aims to raise global awareness of hepatitis B and hepatitis C and encourage prevention, diagnosis and treatment. It has been supported by the World Hepatitis Alliance since 2007 and in May 2010, it got global endorsement from the World Health Organization (WHO, 2011).

2.3 Virology and Genome of HBV

Virus particle, (virion) consists of an outer lipid envelope and an icosahedralnucleocapsid core composed of protein (Figure2.1). 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 and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses, with a diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies. The outer surface contains the HBsAg and surrounds a 27nm innercapsid core that contains HBcAg (Brooks *et al*., 2010).

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; Brook *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 proteinmolecule 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 re-joined (Brook *et. al*., 2010).

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 (HBsAg). The HBsAg gene is one long open reading frame but contains three in frames "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,

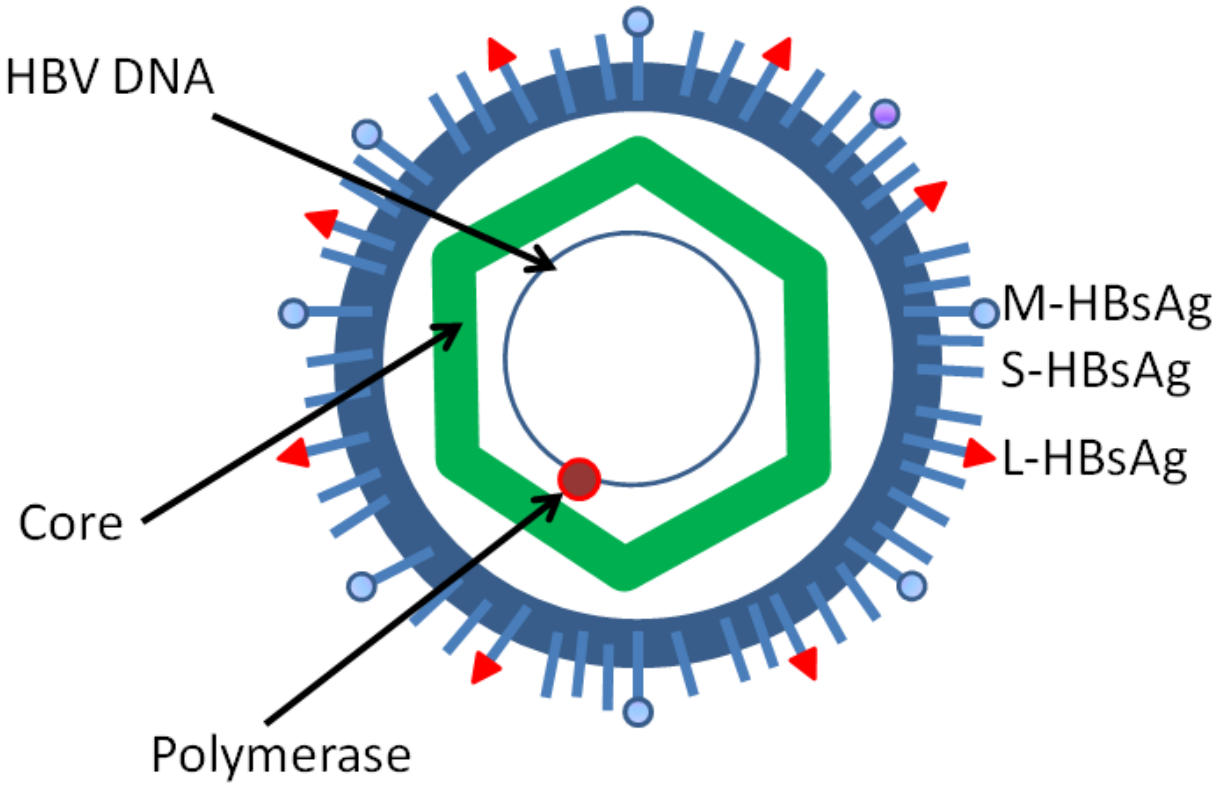


Figure 2.1: The structure of hepatitis B virus
Source: Locarnini, (2004)

medium, 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).

2.4 Transmission of HBV

Transmission of HBV results from exposure to infectious blood or body fluids, through unprotected contact or abrasion (Fairleyn and Read, 2012). Blood transfusions and transfusion with other human blood products, (Buddeberg *et al.,* 2008). Other modes of transmission includedirect percutaneous inoculation of HBV by needles during injection-drug use is an important mode of transmission. Transmission of HBV may also occur by other percutaneous exposure, including tattooing, ear piercing, and acupuncture, as well as needle sticks or other injuries from sharp instruments sustained by medical personnel. These exposures account for only a small proportion of reported cases in the United States. Breaks in the skin without overt needle puncture, such as fresh cutaneous scratches, abrasions, burns, or other lesions, may also serve as routes for entry (CDC, 2012).

Perinatal transmission from mother to infant at birth is very efficient. If the mother is positive for both HBsAg and HBeAg, 70%–90% of infants will become infected in the absence of postexposure prophylaxis. The risk of perinatal transmission is about 10% if the mother is positive only for HBsAg. As many as 90% of these infected infants will become chronically infected with HBV (CDC, 2012).Hepatitis B virus can be transmitted between family members within households, possibly by contact of nonintact skin or mucous membrane with secretions or saliva containing HBV (Shi *et. al.* 2011). However, at least 30% of reported hepatitis B among adults cannot be associated with an

identifiable risk factor.Shi *et. al.* (2011), showed that breastfeeding after proper immunoprophylaxis did not contribute to MTCT of HBV.

It is also known that transmission can occur among intravenous drug users, homosexuals, health care personnel, as well as those who receive frequent blood transfusion such as haemophilic patients. A study done in Barcelona showed that there was a higher prevalence (22.5%) among intravenous drug users, thannon-intravenous drug users the prevalence was (7.4%)(Vallejo *et. al.,* 2008). Other factors reported to be associated with acquisition of HBV include increasing age, male gender, low level of education and history of previous surgery, multiple sexual partners, HIV infection, and non-use of condoms (Bwogi, 2009).

2.5 Replication of HBV

Hepatitis B viruses are DNA viruses characterized by their very small genome size and their unique replication via reverse transcription. The circular genome has been efficiently exploited, thereby limiting genome variation, and leaves no space for genes in addition to those essentially needed during the viral life cycle (Protzer and Schaller, 2000).The replication cycle of HBV is complex(Figure 2.2). It 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 HBV-preS-specific receptors 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

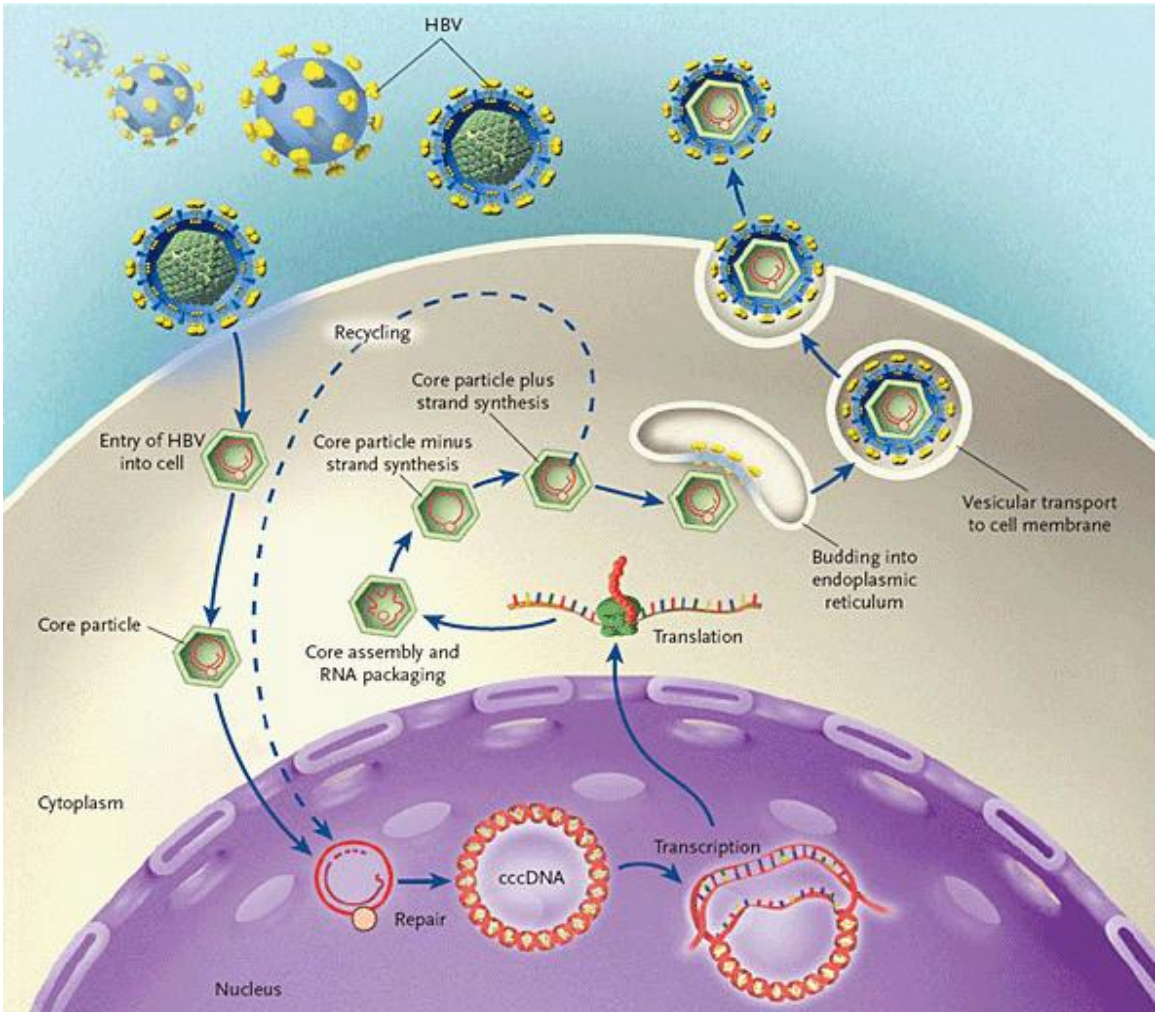


Figure 2.2: The Replication of hepadnaviruses (Ganem and Prince, 2004). HBV virions bindto an as yet unidentified receptor on the hepatocyte surface and are internalised. Thenucleocapsid is released into the cytoplasm and migrates to the nucleus where the partiallydouble stranded DNA genome is converted into cccDNA. This cccDNA serves as templatefor the transcription of the pgRNA and subgenomic mRNAs that are translated in thecytoplasm into the viral proteins. Progeny viral capsids are assembled using the pgRNA,core and polymerase proteins. The pgRNA is reverse transcribed into viral DNA within thecapsid and the mature nucleocapsids either bud into the preGolgi compartment where theyare enveloped and exported from the cell via the endoplasmic reticulum or else the genomeis recycled back into the nucleus to be converted into cccDNA. © 2004 Massachusetts Medical Society.All rights reserved.

Source: Ganem and Prince (2004).

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).

2.6 Serotypes and Genotypes of HBV

The virus is divided into four major serotypes (adrq, adw, ayr, ayw3) 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 more 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, 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 is 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 that of genotypes B and C. The 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).

2.7 Pathogenesis of HBV

Hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. A functional receptorSodium Taurocholate Cotransporting Polypeptide(NTCP) a multiple transmembrane transporter predominantly expressed in the liver. Silencing NTCP inhibited HBV and HDV infection, while exogenous NTCP

expression rendered nonsusceptible hepatocarcinoma cells susceptible to these viral infections. Moreover, replacing amino acids 157–165 of nonfunctional monkey NTCP with the human counterpart conferred its ability in supporting both viral infections.(Yan*et. al.,*2012).There is evidence that the receptor in the closely relate duck HBVis carboxypeptidase D(Glebe and Urban, 2007). The virions bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-specific receptors are expressed primarily 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, in particular virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. The CTLs 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).

2.8 Epidemiology of HBV

The prevalence of HBV carriers varies from 0.1%- 2% in low prevalence areas (United States and Canada, Western Europe, Australia and New Zealand), to 3-5% in intermediate prevalence areas (Mediterranean countries, Japan, Central Asia, Middle

East, and Latin and South America) (Kowdley *et. al*, 2012) to 10 – 20% in high prevalence areas (Southeast Asia, China, sub-Saharan Africa). A systematic review focusing on data in the United States estimated that there are 2.2 million individuals with chronic HBV, two-thirds of who were foreign births (Kowdley *et. al*, 2012).

The wide range in HBV carrier rate in different parts of the world is largely related to differences in the age at infection, which is inversely related to the risk of chronicity. The rate of progression from acute to chronic HBV infection is approximately 90% for perinatally acquired infection (Eng-kiong *et al.,* 2013), 20 – 50% for infections between the age of 1 and 5 years, and less than 5% for adult acquired infection (Wasley *et al.*, 2006). Persons infected with this virus may also develop chronic HBV infection, which can lead to cirrhosis or hepatocellular carcinoma. The likelihood that newly infected persons will develop chronic HBV infection is dependent on their age at the time of infection (Shepard *et. al.,* 2006). More than 90% of infected infants, 25–50% of children infected between 1 and 5 years of age, and 6–10% of acutely infected older children and adults develop chronic infection (i.e., they are HBsAg-positive but negative for immunoglobulin M antibodies to hepatitis B core antigen). Immunosuppressed persons likehaemodialysis patients and persons with human immunodeficiency virus infection are also at higher risk of developing chronic infection (Shepard *et. al.*, 2006).

Because of the inverse association between age and risk of chronic infection, persons infected as children assume a disproportionately large burden of morbidity and mortality attributable to HBV. In countries such as China, Senegal, and Thailand, infection rates are very high in infants, and continue through early childhood. At that stage the prevalence of HBsAg in serum may exceed 25%. In Panama, New Guinea, Solomon Islands, Greenland, and in populations such as Alaskan Indians, infection rates in infants are relatively low and increase rapidly during early childhood(WHO, 2014)

More than 2,000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus. Three quarters of the world's population live in areas where there are high levels of infection (WHO, 2014).

2.9 Immunity of HBV

Hepatitis B viruses are prototype non-cytopathic viruses causing persistent infection. Human hepatitis B virus as well as the closely related animal viruses, more frequently is transmitted vertically from mothers to their offspring (Protzer and Schaller, 2000). Because infection usually persists for many years, if not lifelong, the virus needs efficient mechanisms to hide from the immune response of the host. To escape the immune response, they exploit different strategies. Firstly, they use their structural and non-structural proteins multiply. One of the purposes is to alter the immune response. Secondly, they replicate by establishing a pool of stable extrachromosomal transcription templates, which allow the virus to react sensitively to changes in its microenvironment by up or down regulating gene expression. Thirdly, hepatitis B viruses replicate in the liver which is an immunopriviledged site(Protzer and Schaller, 2000).

In contrast to most viruses, HBV has a peculiar kinetic of replication. While most viruses(for example, Hepatitis C Virus, HIV, influenza and dengue) enter a logarithmic phase of propagation immediately after infection, resulting in febrile symptoms; HBV infection is characterized by a delayed amplification of HBV replication and spread, and acute HBV infection is mainly asymptomatic. Moreover, while viral persistence is frequently associated with low viral load and protein expression (eg, HCV, HCMV), chronic HBV infection is typically characterized by the production of extremely high

quantities of HBsAg and HBeAg. An efficient control of viral infections requires the coordinate action of both the innate and adaptive immune responses (Sarene , 2013).

Innate Immunity against HBV: The innate immunity plays a role immediately after infection and can rapidly recognise pathogen-associated molecular patterns (PAMP) such as viral nucleic acids and viral proteins or tissue damage through pattern-recognition receptors. It induces an antiviral state on infected cells through three major mechanisms, namely (i) by producing type I interferons (IFN), (ii) directing natural killer (NK) cell-mediated killing of viral infected cells, and (iii) stimulating the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-12) which might then induce an efficient triggering of the adaptive immunity (Akira *et al.,* 2006).A limited or even absent activation of innate immunity seems to be the hallmark of acute natural HBV infection. Pro-inflammatory cytokines are often undetectable during the early phases of infection (within the first 30 days) in humans infected with HBV and, when present, their production is of lower magnitude and delayed kinetics compared with HCV and HIV infected patients (Stacey *et al.,* 2009). Innate immunity inhibition in HBV infection can also result from the action of HBV proteins (HBsAg, HBeAg) actively secreted by HBV during its replication cycle. A major cellular component of innate immunity contributing to the initial containment of viral infection is NK cells, which are able to recognize and kill viral infected cells. NK cells are extremely abundant in the normal liver, constituting 30 –40% of intrahepatic lymphocytes (Dunn*et. al.,* 2007).

Adaptive immunity against HBV: The adaptive immune response consists of an intricate web of effector cell types that play important roles in the development of anti-HBV immunity. $CD4^+$ classically referred to as helper T cells are robust producers of

cytokines (Wherry *et. al.,* 2003). They are also required for the efficient development of effector cytotoxic CD8 T cells and B cell antibody production. CD8 T cells are able to recognize virus-infected cells and clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms, thus reducing the levels of circulating virus. B cells can neutralize free viral particles through antibody production to prevent re-infectionand might modulate helper T cell function through their ability to present HBV antigens (Wherry *et al.*, 2003).

HBV-specific $CD4^+$ and $CD8^+$cell-mediated responses become generally detectable immediately after the start of the exponential increase in HBV replication, which follows an initial phase of negative or weakly positive HBV-DNA lasting for about 4- 7 weeks after infection. There are clear differences in the adaptive immunity of patients with resolved HBV or chronic infection. The CTL response to HBV is vigorous, polyclonal, and multi-specific in patients with acute hepatitis who ultimately clear the virus, while it is weak or barely detectable in patients with chronic hepatitis. The inability to control HBV infection and the establishment of chronicity leads to a state of relative collapse of virus-specific adaptive immunity. Experiments performed in animal models of viral infections like lymphocytic choriomeningitis virus have clearly demonstrated that the sustained presence of viral antigens leads to a progressive functional decline of virus-specific CD8 cell responses and ultimately deletion of virus-specific T cells.(Wherry *et al.*, 2003).

Despite the cellular immune response being a major contributor to HBV clearance, humoral responses also play a role in the control of the virus. Its clearance is associated with the production of anti-envelope antibodies and sera with high levels of antiviral antibodies (specific for the viral envelope) can control HBV infection Thus, the coordinated activation of both the cellular and humoral arms of the adaptive immune

response ultimately allows the host to control the infection (Sarene , 2013).

2.10 Diagnosis of HBV

The tests, called assays, for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex.

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 (Karayiannis and Thomas,2009). 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, (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. Therefore most hepatitis B diagnostic panels contain HBsAg and total anti-HBc (both IgM and IgG) ((Karayiannis and Thomas,2009).

Shortly after the appearance of the HBsAg, another antigen called hepatitis B e antigen (HBeAg) will appear (Lok and McMahon, 2007). 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 HBV do not produce the 'e' antigen, so this rule does not always hold true (Lok and McMahon, 2007).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.

If the host is able to clear the infection, eventually the HBsAg 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 HBsAg and the appearance of anti-HBs is called the window period. A person negative for HBsAg but positive for anti-HBs either hascleared an infection or has been vaccinated previously.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Chuand Liaw, 2007). 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 (Chu and Liaw, 2007). Carriers who have seroconverted to HBeAg negative status, in particular 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).

PolymeriseChain 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.Individuals with high viral loads, characteristically have ground glass hepatocytes on biopsy (Zoulim, 2006).

2.11 Treatment of HBV

The hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously (Hollinger and Lau, 2006). Early antiviral treatment may be required in less than 1% of people, 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 andYuen, 2007). Treatment lasts from six months to a year, depending on medication and genotype (Albertiand Caporaso,2011).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As of 2008, there were seven medicines 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) (Dienstag, 2008). 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 person'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).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).

2.12 Clinical Manifestation of HBV

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis.During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations can also occur with both acute and chronic infection(Lok, 2013).

2.12.1 Acute hepatitis: Approximately 70% of patients with acute hepatitis B have subclinical or anicteric hepatitis, while 30%develops icteric hepatitis. The disease may be more severe in patients coinfected with other hepatitis viruses or with underlying liver disease (Liaw*et. al.*, 1998).

2.12.2 Fulminant hepatitis: Fulminant hepatitis is unusual, occurring in approximately 0.1- 0.5% of patients. Fulminant hepatitis B is believed to be due to massive lysis of infected hepatocytes. This explains why many patients with fulminant hepatitis B have no evidence of HBV replication at presentation (Lok, 2013).

The reasons that HBV has a fulminant course in some patients are not well-understood. A case-control study evaluated risk factors for a fulminant course in an outbreak among injection drug users (Garfein*et. al.*, 2004).

2.12.3 Chronic Infection with HBV: Chronic HBV infections can be defined as the presence of HBsAg in the serum of an infected individual for at least six months or as the presence of HBsAg in a patient negative for Immunoglobulin (Ig) M antibodies to the hepatitis B core

antigen (HBcAg) (Shepard *et. al.*, 2006). Approximately 90% of babies perinatally infected with HBV and 30% of children horizontally infected develop chronic infections,

but only 5 - 10% of adult infections become chronic (de Franchis *et. al.*, 2003; Huang *et. al.*, 2006; Yim and Lok, 2006). Most chronically infected individuals present with HBeAg-positive chronic hepatitis B 10-30 years after their initial infection (Fattovich, 2003). Chronic HBV infections can be divided into four phases: immune tolerance; immune clearance (HBeAg positive); inactive carrier (HBeAg negative) and reactivation, although not all individuals pass through every phase (Yim and Lok, 2006).

2.12.3.1 Phase one:In the initial immune tolerance phase, analogous to the incubation period of anacute infection, infected individuals have high HBV viral loads and are positive

For HBeAg (Villeneuve, 2005). Their ALT levels are normal and there is minimal or no liver inflammation. Most of those infected at birth or in early childhood pass through this phase, which can last between one and four decades (De Franchis *et.al.*, 2003; Yim and Lok, 2006). In individuals infected in later childhood or asadults this phase is either very short or completely absent.

***2.12.3.2Phase two*:** Immune clearance phase, can be compared to symptomatic Hepatitis in acutely infected individuals. HBeAg persists along with high HBV viralloads and abnormal ALT levels (De Franchis *et al.*, 2003; Villeneuve, 2005; Yim and Lok, 2006). The immunological response of the host results in active inflammation of the liver tissue, and hence progressive liver damage. There is an increased T cell response to HBcAg and HBeAg resulting in the lysis of infected hepatocytes and flares of aminotransferase levels. The frequency and the severity

of these flares increase the risk of cirrhosis and Hepatocellular Carcinoma developing. The immuneclearance phase culminates in seroconversion from HBeAg to anti-HBe. Thisseroconversion occurs spontaneously at a rate of 5 – 10% per year, but may also result from treatment with interferon or other nucleoside analogs.

***2.12.3.2*Phase three**: inactive carrier state ischaracterised by the absence of HBeAg, the presence of HBsAg and anti-HBe,low or undetectable serum HBV DNA levels (<105 copies/ml), minimal to noliver inflammation and normal ALT levels. In some individuals, this state maypersist indefinitely resulting in a sustained remission and a generally good prognosis (Yim and Lok, 2006).

***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. The 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 (Richman *et al*, 2002). Despite the lower levels of HBV viremia, eAg-negative hepatitis can be difficult to treat; usually requiring indefinite treatment with nucleoside therapy, rare 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 (Richman *et al*, 2002).).

Resolved infection:HBV-infected individuals who have lost eAg 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).

Phase Four: Approximately 1 - 5% of chronically infected individuals progress from theinactive carrier state to the fourth phase of infection, where there is a reactivation of HBV replication leading to HBeAg-negative chronic hepatitis B. HBsAg, anti-HBe and HBV DNA are all detectable in the serum but HBeAg is not, ALT levelsare elevated and there is necro-inflammation of the liver tissue.Annually, approximately 8 - 10% of patients with HBeAg-negative chronic HBVinfections develop cirrhosis compared to 2 - 5% in HBeAg-positive patients(Villeneuve, 2005; Yim and Lok, 2006). Patients with chronic HBV infections and cirrhosis have a five year cumulative risk of 15 - 20% of developing HCC.Additional risk factors for the progression to cirrhosis and HCC include being male (suggesting a tumour genic effect of androgens especially high serumtestosterone levels); older age; alcohol abuse (a six fold higher risk of progressionto cirrhosis); co-infection with HCV, Hepatitis DVirus or HIV; aflatoxin exposure; cigarettesmoking; increased levels of HBV replication and the genotype of the HBVinfection (Fattovich, 2003; Villeneuve, 2005; Chan and Sung, 2006; Yim andLok, 2006).

2.13 Prevention and Control of HBV

Several vaccines have been developed for the prevention of hepatitis B virus infection. These rely on the use of one of the viral envelope proteins (HBsAg). The vaccine was

originally prepared from plasma obtained from people who had long-standing hepatitis Bvirus infection. However, it is now made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine(Libbus and Philip, 2009).

The risk of transmission from mother to newborn can be 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 not earlier than 6 months (24 weeks) (Libbus and Philip, 2009). 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 (HBsAg) 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 (Libbus and Philip, 2009).

Following vaccination, HBsAg may be detected in serum for several days; this is known as vaccine antigenaemia (Martín-Ancel *et al*., 2004). The vaccine is administered in two-, three-, or four-dose schedules to infants and adults, which provides protection for 85–90% of individuals. Protection has been observed to last for 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 (Van and Van, 2007).

Unlike hepatitis A, hepatitis B does not generally spread through water and food. Instead, it is transmitted through body fluids; thus, prevention is the avoidance of such transmission routes such as unprotected sexual contact, blood transfusions, re-use of

contaminated needles and syringes, and vertical transmission during child birth. Infants may be vaccinated at birth(O'Connor, 2008).

Besides the WHO-recommended joint immunoprophylaxis starting from the newborn, multiple injections of small doses of hepatitis B immune globulin (Li *et al.*,2004; Shi *et al.*,2010),or oral lamivudine 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.*,2003; Shi, *et al.*,2010) effectively and safely prevent HBV intrauterine transmission.

2.14 Human Immunodeficiency Virus

Human immunodeficiency virus is a lentivirus and a member of the *Retroviridae*family that causes Acquired Immunodeficiency Syndrome (Douek *et al*., 2009).The virus is a very small and has ribonucleic acid (RNA) as its genetic material. When HIV infects animal cells, it uses a special enzyme, reverse transcriptase, to transcribe its RNA into DNA (Viruses that use reverse transcriptase are sometimes referred to as "retroviruses."). When HIV replicates, it is prone to making small mutations, resulting in viruses that vary slightly from each other (Douek *et. al*., 2009).

This ability to create minor variations allows it to evade the body's immunologic defenses, essentially leading to lifelong infection, and has also made it difficult to develop an effective vaccine(Migueles and Connor, 2010). Making it resistant to medications.A condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive(Migueles and Connor, 2010).

Most untreated people infected with HIV-1 eventually develop AIDS (Migueles and Connor, 2010). These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system (Lawn, 2004). Infection with HIV progresses to AIDS at a variable rate influenced by viral, host, and environmental factors (Lawn, 2004). Most infections will progress to AIDS within 10 years of viral infection, some will progress much sooner, and some will take much longer (Buchbinder *et al*., 1994). Because of a progressive decline in their immunological responses, patients with HIV infection are extremely susceptible to a variety of common as well as opportunistic infections (Arora and Arora, 2005).

2.15 History of HIV

Human immunodeficiency virus types 1 and 2, derived from primate lentiviruses, are the aetiologic agents of AIDS. The illness was first described in 1981, and HIV-1 was isolated by the end of 1983 (Brooks *et. al*., 2010).

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 five young homosexual men in Los Angeles (Brooks *et. al*., 2010). Awareness that a significant epidemic was developing grew as case reports mounted and similar immune deficiency 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. As researchers began to describe the epidemiology and risk factors in a systematic way, many theories emerged regarding the cause of the mysterious disease (Brooks*et. al*., 2010).

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 immunodeficiency virus (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 (Brooks *et al*., 2010).

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) and 3.6% in 2012.

2.16 Structure and Virology of HIV

Human Immunodeficiency Virus is different in structure from other retroviruses. It is around 120 nm in diameter (around 60 times smaller than a red blood cell) and roughly spherical.HIV-1 is composed of two copies of non-covalently linked Positive single-stranded RNA enclosed by a conical capsid comprising the viral protein p24, typical of lentiviruses. Lysine tRNA is the primer of the magnesium dependent reverse transcriptase. It packages two copies of its positive strand, unspliced, 5'guanosine-capped and 3'-polyadenylated RNA genome which is used for strand-transfer-mediated recombination and enables the virus to rapidly evolve under stress or pressure from the environment (Lu *et al.,* 2011). The RNA component is 9749 nucleotides long.This genome bears a 5'cap (Gppp), a 3' poly(A) tail, and many open reading frames (ORFs) (Castelli and Levy, 2002). Viral structural proteins are encoded by long ORFs, whereas smaller ORFs encode regulators of the viral life cycle: attachment, membrane fusion,

replication, and assembly.(Castelli and Levy, 2002). This is in turn surrounded by an envelope of host-cell origin. The single-strand RNA is tightly bound to thenucleocapsid proteins, p6, p7 and enzymes essential to the development of the virion, such as reverse transcriptase andintegrase. The nucleocapsid (p7 and p6) associates with the genomic RNA (one molecule per hexamer) and protects the RNA from digestion by nucleases. A matrix composed of an association of the viral protein p17 surrounds the capsid, ensuring the integrity of the virion particle. Also enclosed within the virion particle are Vif, Vpr, Nef, p7 and viral protease. The envelope is formed when the capsid buds from the host cell, taking some of the host-cell membrane with it. The envelope includes the glycoproteins gp120 and gp41.

As a result of its role in virus-cell attachment, the structure of the virus envelope spike, consisting of gp120 and gp41, is of particular importance (Figure 2.3). It is hoped that determining the envelope spike's structure will contribute to scientific understanding of the virus and its replication cycle, and help in the creation of a cure.The first model of its structure was compiled in 2006 using cryo-electron tomography and suggested that each spike consists of a trimer of three gp120–gp41 heterodimers (Zhu *et al.,* 2006).However, published shortly after was evidence for a single-stalk "mushroom" model, with a head consisting of a trimer gp120s and a gp41 stem, which appears as a compact structure with no obvious separation between the three monomers, anchoring it to the envelope. There are various possibilities as to the source of this difference, as it is unlikely that the viruses imaged by the two groups were structurally different(Zanetti, *et. al.,* 2006).

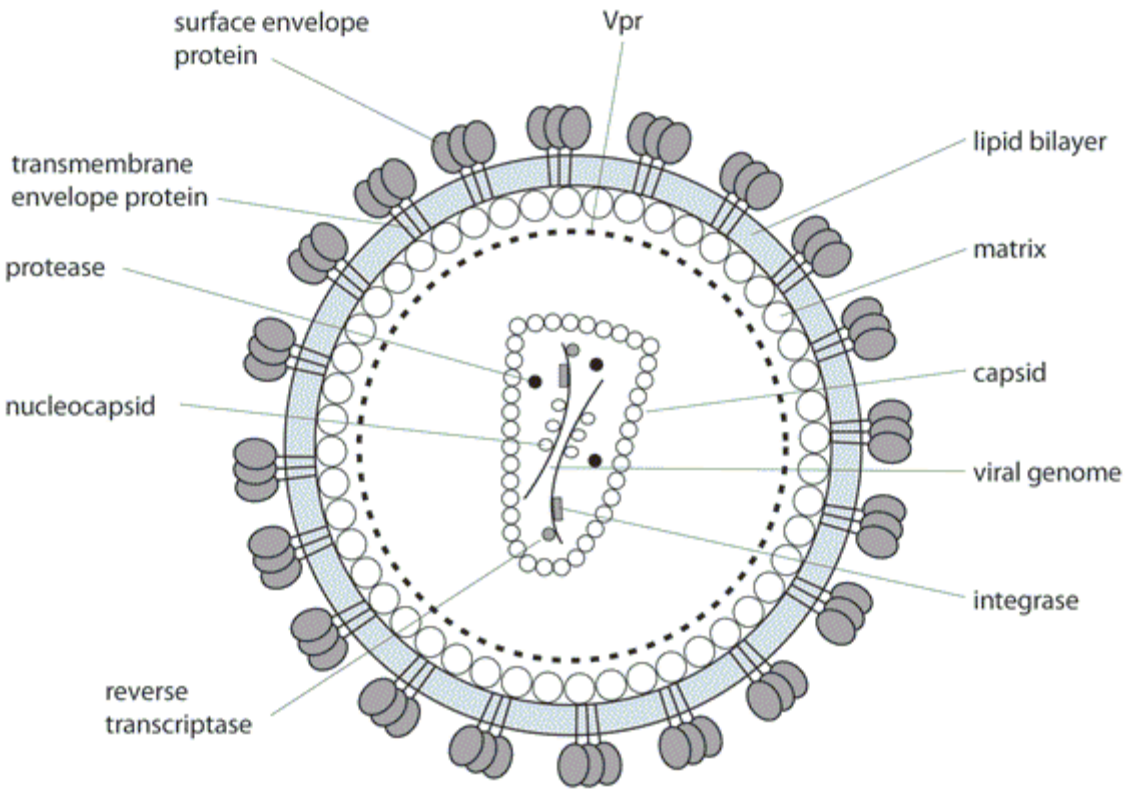


Figure 2.3. HIV Virion

Source: Study material © London School of Hygiene and Tropical Medicine

2.17 Transmissionof HIV

Infection with HIV occurs by contact with blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these body fluids, the virus is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unprotected sex, contaminated needles, breast feeding, and transmission from an infected mother to her baby at birth. Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world (Boily *et al*., 2009).

Heterosexual transmission remains the dominant mode of transmission and accounts for about 85% of all HIV-1 infections. The HIV epidemic is expanding worldwide with an increasing number of distinct viral subtypes and circulating recombinant forms (CRFs). Out of 34 million adults living with HIV and AIDS, women account for one half of all HIV-1 infections worldwide. These gender differences in HIV pathogenesis may be attributed to sex hormones.

According to UNAIDS (2012) report on the global AIDS epidemics, people who inject drugs are among the population groups most severely affected by HIV infection. In virtually all countries reporting data in 2012, the prevalence of HIV infection is higher among people who inject drugs than among the general population that usually get it through other route. In 49 countries with available data, the prevalence of HIV infection is at least 22 times higher among people who inject drugs than for the population as a whole, with prevalence at least 50-fold higher in 11 countries (UNAIDS, 2012). A 2007 study estimated that about 16 million people inject drugs globally, including many younger than 25 years and 3 million of whom are living with HIV (UNAIDS, 2012). In

addition to imposing extraordinary burden on people who use drugs, drug-related transmission also undermines global efforts to the foundation for the eventual end of AIDS. In Eastern Europe and Central Asia, one of two regions where the number of people newly infected is rising, national epidemics are typically driven by drug use–related transmission and by further transmission to the sexual partners of people who use drugs (UNAIDS (2012).

Low and middle-income countries, however, have had limited progress in slowing the spread of HIV among people who inject drugs. Nevertheless, transmissions have been reduced substantially. Such countries as Australia and the United Kingdom that have implemented evidence-informed HIV prevention strategies have sharply reduced the number of people who inject drugs who acquire HIV infection, with some approaching the elimination of drug-related transmission UNAIDS (2012).

A defining feature of the pandemic in the current decade is the increasing burden of HIV infections in women (Quinn and Overbaugh, 2005), which has additional implications for mother-to-child transmission. The transmission of the virus from the mother to the child can occur *in utero* (during pregnancy), intrapartum (at childbirth), or via breast feeding. In the absence of treatment, the transmission rate between conception and time of birth increases to about 25% (Coovadia, 2004). However, where combination antiretroviral drug treatment and Caesarian Section are available, this risk can be reduced to as low as 1% (Coovadia, 2004). It is estimated that 430,000 children were infected worldwide in 2008 (19% of this was new infections), primarily by this route, and that a further 65,000 infections were averted through the provision of antiretroviral prophylaxis to HIV-

positive women (UNAIDS, 2009), hence in 2010, an estimated 370,000 children were infected (UNAIDS, 2010).

The risk of HIV transmission through infected blood products exceeds that of any other risk exposure. Ninety percent of recipients transfused with HIV antibody-positive blood were found to be HIV infected at follow-up (Donegan, *et. al.*, 1994).

2.18 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 of R5 to X4 variants in a given patient, but AIDS can clearly develop without this switch (Sell and Max, 2001). (additional points)

2.19 Methods of subversion of the Immune DefenseMechanisms by HIV

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.

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 these viral antigens (Sell and Max, 2001).

Downregulation of MHC:Viral products, such as Nef (The accessory Nef protein allows human immunodeficiency virus type 1 (HIV-1) to persist at high levels and to cause AIDS in infected humans) 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 ability/differentiation 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 requiresCD4 T cell help. With the loss of this help, the numbers of CD8 T cytotoxic cells decrease.

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 have 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 1recognition and without killing the infected cells (Sell and Max, 2001).

2.20 Pathology of HIV

Human Immunodeficiency 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 theCluster of Differentiation 4 ($CD4^+$ - is a glycoprotein found on the surface of immune cells such as T-helper cells, monocytes, macrophages, and dendritic cells.)lymphocyte count in the blood(FMOH, 2010). The $CD4^+$ tests are normally reported as the number of cells in a cubic millimeter of blood, or mm3 (Cunningham *et al*., 2010). 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.

Human immunodeficiency virus infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$), macrophages, and dendritic cells (Cunningham *et al*., 2010). The viral infection leads to low levels of $CD4^+$through three main mechanisms: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells and thirdly, killing of infected $CD4^+$by CD8 cytotoxic lymphocytes that recognize infected cells (Cunningham *et. al*., 2010). When $CD4^+$numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infection.There is some disagreement about the normal range for $CD4^+$ counts, but normal $CD4^+$ counts are between 500 and 1600, and CD8 counts

are between 375 and 1100 (Cunningham *et al*., 2010). The $CD4^+$counts drop dramatically in people with HIV, in some cases down to zero. Recent research has indicated that it may be easier to maintain higher $CD4^+$ counts if HIV treatment starts before $CD4^+$ counts drop below 350 cells/mm3 (Bartlett and Gallant,2010).

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 $CD4^+$ reduction lead to progressive immune dysfunction, eventually resulting in clinical disease progression (Brooks *et al*., 2010). The progression of HIV infection is determined by the relationship of the rate of reproduction of virus to the production of new $CD4^+$(Brooks*et. al*, 2010). During latency, it is estimated that 100 billion new viral particles are being produced every day and about 2 billion $CD4^+$ are dying and being regenerated everyday (Brooks *et. al*., 2010).

2.21 Pathogenesis of HIV

The lentivirus HIV causes AIDS by interacting with a large number of different cells in the body and escaping the host immune response against it. The steps occurring in infection involve an interaction of HIV not only with the $CD4^+$ molecule on cells but also with other cellular receptors recently identified (Cunningham *et al*., 2010). Virus-cell fusion and HIV entry subsequently take place. HIV strains are highly heterogeneous, and certain biologic and serologic properties determined by specific genetic sequences can be linked to pathogenic pathways and resistance to the immune response (Cunningham *et al*., 2010).(addition)

2.22 Epidemiology

The Acquired Immunodeficiency Syndrome is a major health problem in many parts of the world, and is considered a pandemic, a disease outbreak that is not only present over a large area but is actively spreading. According toWHO(2014) report, some 35 million people have been infected worldwide, with some 1.5 million deaths out of which 24,000 were children. The UNAIDS (2009) reported that, at the end of 2010, 34 million people were globally living with HIV, 2.7 million were newly infected and 1.8 million died of AIDS related illness. The region most heavily affected remains sub-Saharan Africa with South Africa having more people living with it (about 5.6 million). According to the 2010 seroprevalence sentinel survey, the prevalence rate of HIV in Nigeria is 4.1% with Kaduna state having 5.1 % Ref. Women were more susceptible to HIV-1 infection. Susceptibility of womenmight be due to hormonal changes, vaginal microbial ecology and physiology, and a higher prevalence of sexually transmitted diseases as postulated bysSagar *et al*.(2004) and by Lavrey*et al*.(2004).

According to WHO/UNAIDS/UNICEF (2011), the global incidence of HIV infection has stabilized and on the decline in many countries with generalized epidemics. The number of people receiving antiretroviral therapy continues to increase with 6.65 million people getting treatment at the end of 2010. New surveillance data confirm that the epidemic disproportionately affect sex workers, men who have sex with men, transgender people, people who inject drugs, prisoners and migrants in both concentrated and generalized epidemics (WHO/UNAIDS/UNICEF (2011).

2.23 Immunity toHIV

The likelihood of productive HIV infection may not just be a stochastic event after one is exposed. Several lines of evidence suggest that several immune parameters may play a role in the preferential infection of particular target cells by HIV; these include the expression of particular immune markers on the surface of cells, the differentiation subset of target cells, and the activation state of particular cell types (McKinnon and Kaul, 2012) Human immune deficiency virus infected persons develop both humoral and cell mediated responses against HIV related antigens. Antibodies to a number of viral antigens develop soon after infection (Brooks *et al*., 2010).

2.21.1 Innate immunity

The first line of defenseagainst infection is mediated by the innate immune system. This system includes external barriers such as skin and mucosal surfaces, but also biologicallysynthesised products and cells that act in a non-pathogen-specific manner against invading microbes. Cells of the innate immune system are responsible for localized host responses that act within hours of infection. These cells also capture and transport foreign materials from the peripheral surfaces of the body to the lymphoid organs and there subsequently present the foreign material to the adaptive immune system (Trombette and Mellman, 2005).

Although historically the innate immune system is termed non-specific, it recognizes certain pathogen-specific patterns (e.g. proteins, DNA or RNA sequences and sugar motifs) via the different pathogen recognition receptors (PRRs) like Toll like

receptor(TLRs) and mannose receptors. (Medzhitov, 2007). The innate immune responses are more or less a requirement for induction of adaptive immunity.

The cells of the innate immune system can directly contribute to defense against HIV-1 by secretion of antiviral soluble factors. For instance, increased levels of IFN- , tumour necrosis factor (TNF) and β-chemokines such as RANTES, MIP-1α and MIP-1β have been suggested to contribute to the resistance against HIV-1 infection in individuals who are continuously exposed to HIV-1 but remain uninfected (Montoya *et al.,* 2006a). The beneficial effect of the β-chemokines may be explained by their ability to bind the CCR5 receptor on cells and thereby inhibit the binding and entry of the virus (Cocchi *et al.,* 1995). Other anti-HIV components include proteins involved in the complement system and a variety of cytokines such as type-1 IFN-α (Fitzgerald-Bocarsly *et al.,* 2008) that counter-acts HIV-1 replication, by indirect inhibition of viral replication and activation of NK- and T cells (Montoya *et al.,* 2006a). An intracellular protein, tripartite motif protein 5α(TRIM-5α) has been attributed to restrict HIV-1 replication in old worldmonkeys. By binding to the viral capsid, TRIM-5α interferes with the uncoating process, therefore preventing successful reverse transcription. However, HIV-1 can infect both NK cells and DCs and thereby interfere with their antiviral activities(Harada *et. al.,* 2007).

2.21.2 Adaptive immunity

The adaptive immune response is highly specific and is generally effective in combating infections. In contrast to innate immune system the development of an adaptive response is relatively slow. It usually takes several days or weeks to develop following

anencounter with a new pathogen. The two main arms of the adaptive immune response are the cellular and humoral arms, mediated by T and B cells, respectively. Both cells types originate from the same haematopoietic stem cells in the bone marrow (Harada *et al.,* 2007).

2.21.3 Cellular immune response

The adaptive cellular immune response includes two cell types, the primarily B cells/ T cells. The T cells consist of two principal cell types CD4 T and CD8 T cells. There are other T cells.Cell types are the $CD4^+$ T helper cells and the cytotoxic $CD8^+$. $CD4^+$plays a central role in directing and regulating many processes of the adaptive immune response. They recognize peptide antigens, derived from extracellular pathogens which are presented by human leukocyte antigen (HLA) class II molecules on professionals APCs. There are several different subsets of $CD4^+$, including T-helper (Th) 1, TH2, Th17 and regulatory T cells. One important function of the various T helper cells is to provide support for the other immune cells (Bettelli *et. al.,* 2008).Importantly, T helper cells secrete cytokines and express co-stimulatory molecules to promote differentiation and maturation of B cells. Th1 mediated immune responses have been attributed to control intracellular pathogens, whereas Th2 cells help in clearing extra cellular pathogensand can cause tissue inflammation and in some cases autoimmunity (Fouser *et. al.,* 2008). The Th17 cells are preferentially lost in the gastrointestinal tract during acute HIV-1 infection (Brenchley*et. al.,* 2008). Regulatory T cells are essential for maintaining peripheral tolerance, preventing autoimmune diseases and limiting chronic inflammatory disease (Vignali *et. al.* 2008).

The $CD8^+$ T cells or cytotoxic T lymphocytes (CTL) are the main effectors of the cellular immune system, with the objective of eliminating infected cells (Sandberg *et. al.,* 2001). CD8T cells recognize peptide antigens derived from intracellular pathogens presented on HLA class 1 molecule of the infected cells. Cytotoxic T cells have the potential to recognize and kill any infected cells that express HLA class 1 molecules. One way for the CTL to induce apoptosis is by granule-mediated cytotoxicity. This mechanism requires the interaction between the MHC class 1 molecule and the T cell receptor that result in the release of cytotoxic granules from the CTL. The release of pro-apoptotic enzymes, including perforin and granzymes from the granules, induces apoptosis in the target cell (Voskoboinik, *et. al.*, 2006). Induction of apoptosis in target cells can also be mediated by the $CD8^+$T cells which can bind toFas (CD95) molecules on the target cells.

Furthermore, ccytotoxic T cells can also secrete high amounts of the antiviral cytokine IFN-□, which can block viral replication and induce up-regulation of MHC molecules on the surface of infected cells, resulting in more efficient antigen presentation. IFN-□ can also activate macrophages, NK cells and promote isotype switching in B cells. As mentioned above, the control of virus replication during the acute phase of HIV-infection has been largely attributed to the effector functions of $CD8^+$ T cells (Almeida *et. al.*, 2007).

2.21.4. B Cells and Induction of Humoral Immune Responses

B cells are professional antigen presenting cells that can evolve into antibody producing plasma B cells. The induction of the humoral immune response requires an antigen

(soluble or membrane bound) that is recognized by membrane-bound immunoglobulin (Ig) on the surface. The antigen is taken up by the B cell and processed into peptides, which are displayed on the cell surface with HLA class II molecules (Gudmundsdotter, 2009). The TCR and the $CD4^+$ T cell molecules on the T helper cell recognize the foreign epitode and subsequently provide co-stimulatory signals and IL-4. In this manner the T helper cells stimulate the maturation of the naïve B cells into fully differentiated antibody secreting plasma B cells. They also stimulate isotype antibody switching from IgM or IgD to IgG, IgA and IgE. Antibodies can facilitate protective immunity by inducing antibody dependent cytotoxicity (ADCC) or by direct neutralization of the virus (Gudmundsdotter, 2009).

HIV-1 is unable to infect B cells because of the lack of $CD4^+$ expression on the B-cell surface. However, HIV-infection is associated with several B-cell abnormalities such as B cell hyper-activation due to unspecific stimuli of microbial products binding to TLRs, or bystander activation and phenotypic alterations. The mechanism by which HIV-1 impairs humoral immunity may either be direct virus induced B cell defects, or polyclonal B cell activation(Moir and Fauci, 2008).

2.24 Treatment of HIV

There is currently no cure for HIV infection, though the search is on and many drugs are at various stages of testing. Antiviral drugs currently approved for use according to Willey *et. al.* (2011) are:

1. **Nucleoside reverse transcriptase inhibitors (NRTIs):**Examples include Zidovudine (AZT or Retrovir), didanosine (Videx), zalcitabine (ddc orHIVID),

tenofovir (Viread), emtricitabine (Emtriva or Coviracil), stavudine (Zerit), and lamivudine (Epivir or3TC).

2. **The non-nucleoside reverse transcriptase inhibitors (NNRTIs):**This includes delavirdine (Rescriptor), efavirenz (Sustiva), and nevirapine (Viramune).
3. **The protease inhibitors (PIs):** Examples include indinavir (Norvir), nelfinavir (Viracept), lopinavir (Kaletra), Fosamprenavir (Lexiva), atazanavir (Reyataz), and saquinavir (Invirase).
4. **The fusion inhibitors (FIs):** Example is enfuvirtide (Fuzeon). "Therapy with Highly Active Antiretroviral Therapy" (HAART) became available in 1996; it often can suppress viral replication to below limit of detection in plasma, decrease viral loads in lymphoid tissue, allow the recovery of immune response to opportunistic pathogens, and prolong patient survival (Brooks *et. al*., 2010).The first HIV-inhibitor Zidovudine (AZT) was approved for use in America in 1987. Since then more agents of the same class, i.e. reverse transcriptase inhibitors (NRTI) have become available leading to the use of duo therapy. In the early nineteen-nineties, duo therapy was more effective than AZT monotherapy (Hoepelman*et. al.,* 2009). During this early nineteen-ninety's agents of second class of treatment, the non-nucleoside transcriptase inhibitors (NNRTIs) became available. The use of these drugswas limited due to the rapid loss of effect caused by the development of resistance. The introduction of a third class, the protease inhibitors (PIs), in 1996 resulted in the use of a combination therapy consisting of three drugs of two separate classes, leading to a durable effect.(Hoepelman*et. al.*, 2009). In industrialized countries, these combinations, since then referred to as highly-active antiretroviral therapy (HAART) became a treatment standard for naïve (previously untreated) HIV-infected patients and resulted in a tremendous decline in HIV and AIDS-related morbidity and mortality (Hoepelman*et. al.*, 2009).

Treatment with HAART is not curative.Continuous, lifelong treatment with antiretroviral therapy has significantly improved life expectancy and turned HIV from a terminal infection into a more chronic disease. Despite the success of HAART, the risk of development of drug resistance that may lead to complete treatment from HIV without adverse side effect is a continuous worry in the era of HAART.The presence of drug resistance limits future treatment options because of cross-resistance. Incomplete adherence has been found to be the most common cause of virological failure (Robbins *et. al.,* 2007).

Unfortunately, HAART is associated with the occurrence of considerable number of adverse events. Chronic use of HAART can lead to significant health problems, such as diabetes, cardiovascular disease and lipohypertrophy or lypoatrophy syndrome. The latter condition leads to changes in body shape which might have psycho-social consequences(Hoepelman *et. al.,* 2009).

Since the introduction of HAART, progress has been made in the field of HIV-treatment. The introduction of newer agents with differing resistance profiles into the existing antiretroviral classes and of two new classes-entry inhibitors and integrase inhibitors and of longer-acting agents has led to simpler treatment options and to new options for the treatment of multi-drug resistant viruses (Kozai, 2009; Wilson and Gallant, 2009).

In 1996 HAART consisted of a combination of many pills (12-18 per day) that had to be taken twice (every 12 hours), or even three times a day (every 8 hours) with food prescriptions that need to be observed, nowadays most HIV-infected persons start with a once-daily regimen for some of them this regimen consists of only combination of pill

per day. Although most naïve patients need starts with a simple regiment and most of the treated patients switch to a simplification of their regimen, a growing number of the treated patients and of naïve patients need more complex regimens based on specific drug resistance profiles this is due to increase in baseline resistance over recent years (Hoepelman*et. al.*, 2009)

2.25 Diagnosis of HIV

The diagnosis of HIV-1 infection is based on the detection of specific antibodies, antigens, or both, and many kits have been developed and are available commercially. Testing for the viral antibodies is the only way to know whether one has been infected. However, the HIV antibody test only works after the infected person's immune system develops antibodies to it (UCSF 2011). During the "window period", between the initial infection and the period in which antibodies are detectable (which can be from 2 weeks to 6 months, but is usually around 3 months), standard HIV antibody testing may be negative, even though a person is infected it is too early for the antibody test to be positive. However, it can be diagnosed during this window period by detection of viral nucleic acid or viral tissues (UCSF 2011).

Serological tests are generally used for screening. A major advance has been the availability of rapid HIV-1 antibody tests. These assays are easy to perform and provide results rapidly (Greenwald *et. al*., 2006), enabling specimen collection and proper diagnosis at the same visit.

Rapid tests are important tools for surveillance, screening, and diagnosis, and can be reliably done on plasma, serum, whole blood, or saliva by health-care providers with little

laboratory expertise. The two limitations of these serological tests are detection of infection during primary infection when antibodies are absent, and in infants younger than 18 months who might bear maternal HIV-1 antibodies. In these instances direct virus detection is the only option (e.g., quantification of viral RNA or p24 antigen in heat denatured serum. For staging purposes, measurement of $CD4^+$T cells and viraemia is required (Greenwald *et. al*., 2006).

Plasma viral load is widely used to monitor therapeutic success of antiretroviral treatment. Several commercially available tests provide sensitive quantification of plasma HIV-1 RNA copies. The newer versions of the Amplicor and Quantiplex (Roche, Indianapolis, IN, USA, and Bayer Diagnostics, Walpole, MA, USA, respectively) assays have overcome initial suboptimum performance for non-B subtypes (Berger *et al*., 2005). While the viral load determines the rate of destruction of the immune system, the number of $CD4^+$ cells reveals the degree of immunodeficiency and is, therefore, used to assess the stage of infection. The $CD4^+$T cell counts together with clinical manifestations (e.g., occurrence of opportunistic infections) are key criteria for HIV-1 disease classification.

Flow cytometry analysis is the standard method for $CD4^+$T cells quantification. Standard methods for quantifying viral load and $CD4^+$T cell counts need advanced laboratory infrastructures, and assays require a specimen to be tested within a short time of collection. These requirements, pose challenges for resource-constrained settings. The use of dried blood spot specimen has resolved some of the difficulties associated with transportation of samples needed for virological assessments (Uttaymakul *et al*., 2005). Similarly microcapilliary flow-based systems, $CD4^+$ T cells chips, or total white counts (panleucocytegating) provide alternatives for establishment of the level of

immunodeficiency in resource-limited settings (Ceffa *et. al*., 2005; Rodriguez *et. al*.,2005). However, people at risk for HIV infection should have regular testing to ensure early diagnosis of this infection, as many treatment options are available (Adam 2013).

2.26 Symptoms of HIV

Infection with HIV generally occurs by introduction of bodily fluids from an infected person into the body of an uninfected person. A period of rapid viral replication ensues, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood (Piatak *et al*., 1993). This response is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells. Thus acute viremia is associated in virtually all patients with the activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ counts rebound(Piatak *et al*., 1993).

A good $CD8^+$ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo *et al*., 1997). During this period (usually 2–4 weeks post-exposure) many individuals develop an influenza or mononucleosis-like illness called acute HIV infection, the most common symptoms of which may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophageal sores, and may also include, but less commonly, headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms(Pantaleo *et. al*., 1997).

Infected individuals may experience all, some, or none of these symptoms.These symptoms can occur within days or weeks of the initial exposure to the virus during a period called primary or acute HIV infection (UCFS, 2011). Many infections that are not HIV can cause similar symptoms, including mononucleosis, viral hepatitis, other sexual transmitted infections, and viral hepatitis. Stress and anxiety can also produce similar symptoms in some people, even though they do not have HIV (UCFS, 2011).

The duration of symptoms varies, averaging 28 days and usually lasting at least a week (Kahn and Walker, 1998). Because of the nonspecific nature of these symptoms, they are often not recognized as signs of HIV infection. Even if patients go to their doctors or a hospital, they will often be misdiagnosed as having one of the more common infectious diseases with the same symptoms. As a consequence, these primary symptoms are not used to diagnose HIV infection, as they do not develop in all cases and because many are caused by other more common diseases. However, recognizing the syndrome can be important because the patient is much more infectious during this period (Daar *et al*., 2001).

2.27 Chronic Infection

A strong immune defense reduces the number of viral particles in the blood stream, marking the start of secondary or chronic HIV infection. The secondary stage of HIV infection can vary between two weeks and 20 years. At this phase of infection, HIV is active within lymph nodes, which typically become persistently swollen, in response to large amounts of virus that become trapped in the follicular dendritic cells (FDC) network. The surrounding tissues that are rich in $CD4^+$ T cells may also become infected, and viral particles accumulate both in infected cells and as free virus. Individuals who are

in this phase are still infectious; $CD4^+$,$CD45RO^+$ T cells carry most of the proviral load (Clapham and Mcknight, 2001).

2.28 Acquired Immune Deficiency Syndrome

The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are opportunistic infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages (Kallings *et al*., 2003). These infections affect nearly every organ system. People with AIDS also have an increased risk of developing various cancers such as Kaposi's sarcoma, cervical cancer and cancers of the immune system known as lymphomas. Additionally, people with AIDS often have systemic symptoms of infection like fevers, sweats (particularly at night), swollen glands, chills, weakness, and weight loss (Guss, 1994). Opportunistic infection usually do not occur in HIV-infected patients until CD4 T cell count have dropped from normal level of about 1000 cells/µl to less than 200 cells/µl(Guss, 1994).

The most common opportunistic infection in untreated AIDS patient reported by Brooks *et al.* (2010) includes:

- **Protozoa**: *Toxoplasma gondii, Isospora belli, Cryptosporidium*.
- **Fungi**: *Candidaalbicans, Cryptococcus neoformans, Coccidiodes immitis Histoplasma capsulatum, Pneumocystis jiroveci.*
- **Bacteria**:*Mycobacterium, avium-intracellulare, M. tuberculosis, Listeria Monocytogenes, Nicardia asteroids, Salmonella species, Streptococcus.*
- **Viruses**: *Cytomegalovirus, Herpes simplex virus, Varicella-zoster virus, Adenovirus, Polyomavirus, JC virus, Hepatitis B virus, Hepatitis C virus.*

Diarrhoea is one of the most common presenting complaints in HIV-infected individuals. The infectious etiological agents include both opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non-opportunistic agents that usually cause acute, treatable diarrhoeal illness(Alemu *et.al*. 2011). Chronic diarrhoea, defined as persistence of diarrhoea beyond four weeks is a common symptom in HIV-infected patients in the tropics. Due to the delayed diagnosis of HIV in these individuals, the patients usually take over-the-counter drugs or local medications for symptomatic relief and the underlying disease is left untreated. The World Health Organisation defines diarrhoea wasting syndrome along with a positive HIV serology test to be an AIDS-defining illness (Alemu *et. al*., 2011).

2.29 Prevention of HIV

Prevention and control of AIDS is achieved primarily through education (Willey *et al*., 2011). The health education for the general public has been summarized as follows (Brooks *et al*., 2010):

- Any sexual intercourse (outside of mutually monogamous HIV antibody-negative relationships) should be protected by a condom.
- Do not share unsterile needles or syringes.
- All HIV-infected mothers should avoid breast feeding to reduce transmission of the virus to their children if safe alternative feeding options are available.

Population Action International in 2008, reported that even before the term 'ABC' was conceived, materials provided by WHO, the Global Program on AIDS (later succeeded by UNAIDS) and governments and organizations around the world,

contained information on how abstinence, fidelity and condom use could prevent the sexual transmission of HIV. According to UNAID's 2004 Global Report on the AIDS Epidemic, 'ABC' stands for:

- Abstinence (not engaging in sex, or delaying first sex)
- Being safer, by being faithful to one's partner or reducing the number of sexual partners.
- Correct and consistent use of condoms.

The following are broader strategies for preventing HIV among children as reported by WHO (2013),

- Preventing HIV infection among prospective parents by making HIV testing and other prevention interventions available in services related to sexual health such as antenatal and postpartum care and focusing on preventing HIV in women of child-bearing age.
- Avoiding unwanted pregnancies among HIV positive women by providing appropriate counselling and support to women living with HIV to enable them to make informed decisions about their reproductive lives. Ensuring that contraception is available to women who want it.
- Preventing the transmission of HIV from HIV positive mothers to their infants during pregnancy, labour, delivery and breastfeeding.
- Integration of HIV care, treatment and support for women found to be positive and their families.

2.30 Hepatitis B and HIV Co-infection

Markers of HBV exposure are present in a high proportion of HIV-infected individuals (Hoffman and Thio 2007). Human Immunodeficiency Virus affects HBV viral replication and clearance, accelerates the development of liver disease, and contributes significantly to hepatic morbidity and mortality in HIV infection (Hoffman 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; Hoffman and Thio 2007). Factors affecting the prevalence of chronic HBV include age at time of infection and mode of acquisition, which vary geographically (Hoffman and Thio 2007).

In the United States and Western Europe, HBV often is often 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 (Alter 2003). 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 2003). In the United States, HIV/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 are the most common modes of transmission of the viruses. Overall HBV prevalence is higher; the prevalence of HBV among HIV-infected individuals is also higher, at an estimated 20-30% (Uneke *et al*., 2005 and Hoffman *et al*., 2007).

Human immunodeficiency 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 immunodeficiency 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(Peter, 2007).

It has been recommended that 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 HBsAg -positive, hepatitis B core antibody (anti-HBc total)-positive and anti-HBs-positive status (Peter, 2007). 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).

Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as a result of highly active antiretroviral therapy (HAART), liver diseases due to chronic HBV infections become a leading cause of death. The complex interactions between HIV-HBV co-infection and HAART are increasinglyapparent in HIV disease progression (Wondimeneh, *et. al.,* 2013).In HIV-HBV co infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and increased risk of hepatocellular carcinoma at lower $CD4^+$ counts.

The impact of HBV could not be limited in causing liver hepatotoxicity but also results in failure in immunological recovery in HIV positive patients. For example, a study in Tanzania reported slow rate of immunologic recovery after initiation of HAART treatment and higher risk of hepatotoxicity among HIV/HBV co-infected patients (Christian *et al.,* 2010). Thus the management of HBV in HIV infection is complicated and brings high burden in particular where HIV is rampant.Therefore, globally HIV, HBV become the major public health concerns(Wondimeneh*et. al.,* 2013). In some countries, screening of HIV-infected individuals for HBV is highly recommended before initiation of antiviral treatment .In Ethiopia; the seroprevalence of HBV among HIV positive individuals is scarce except few reports among blood donors and infection prone groups (Wondimeneh*et. al.,* 2013). In addition, there is no report about liver enzyme levels and $CD4^{+}$T cell count determination among HIV-HBV co infected patients. (Wondimeneh *et. al.*, 2013).

2.31 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 coinfection have higher levels of HBV DNA and lower rates of clearance of the hepatitis B e antigen (HbeAg). Serum transaminase levels may be lower in HIV/HBV-coinfected 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 immunodeficiency 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 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%). also, 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/HBV treatment. They also can occur spontaneously (Bonacini *et al*., 2004; Weber 2006).

2.32 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 counselled to avoid or limit intake of hepatotoxins, including alcohol and high dosages of acetaminophen (Firnhaber and Ive 2009; Luetkemeyer2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Ahmadu Bello University(ABU), Zaria. This is one of the first Generation University in the Northern part of Nigeria. It is 81 kilometers from the state capital, Kaduna and about 300 km from Abuja the federal capital of Nigeria. Zaria is in Northern Guinea Savanna and lies which lies onlongitudes$11^{0}03^{/}$N; and $07^{0}42^{/}$E. It covers a land area of about 7,000 hectares. The University has 12 Faculties, a school of Postgraduate Studies and 95 academic Departments. There are also six specialized Centers and a Division of four Agricultural Colleges. This is the largest and the most extensive of all Universities in sub-Saharan Africa (School of Postgraduate Studies Handbook, 2011).

3.2 Study Design

This study was a cross-sectional study which involved male and female students from different faculties.The participants' consent was sought and blood samples were

collectedfrom consecutive consentingstudentsaged 16 years old and above for examination. The University Health Services Centre was used as the collection point.

3.3 Study Population

The target population included undergraduate and postgraduate students of Ahmadu Bello University, Zaria. The student body is over 30 thousand, majority of which are undergraduates but with a sizeable and growing body of postgraduate students.

3.4 Ethical Clearance and Consent

Ethical clearance (Appendix I) was obtained from the Medical Ethical and Scientific Research Committee of Kaduna State Ministry of Health, before the commencement of work. Consent form was given after explanation to obtain permission of the student. Afterward which the students signed as a sign of agreement to participate in the study.(Appendix II).

3.5 Inclusion Criteria

All undergraduate and postgraduate students of ABU, Zaria who gave their consent to participate in the study.

3.6 Exclusion Criteria

Non students and students who did not give their consent to participate in the study.

3.7 Sample size

A sample size was determined using the equationas described by Naing *et al.* (2006)

$$n = \frac{z^2pq}{d^2}$$

Where:

n = Sample size

z = Standard normal deviation at 1.96 (which corresponds to 95% confidence interval)

p= Prevalence rate of 12.5% froma previous study of HBsAg)(Aminu*et. al.*, 2013)

q = 1-p; and

d = degree of accuracy/precision expected (allowable error) 5% = 0.05.

Thus, $n = \frac{(1.96)^2 \times 0.125(1- 0.125)^2}{(0.05)^2}$

$$\frac{(0.4802)\ (0.765625)}{0.0025}$$

n= 147.06125

n = 147

The sample size calculated was 147. However, a total of 600 samples werecollectedfor the study.

3.8 Questionnaire Administration

A structured questionnaire (Appendix III) was administered to the consenting students in order to obtain information on their socio-demography, clinical data and potential risk factors that might be associated with HBV and HIV infection.

3.9 Sample Collection and Processing

The seroprevalence of HBsAg and HIV among students of Ahmadu Bello University, Zaria was determined between Aprils to August, 2013. Three milliliters of venous blood sample was collected from each of the consenting students using clean labelled sample bottles by the Laboratory Scientist. To obtain serum, each blood sample was separated out by centrifugation at 1000rpm (revolutions per minute) for 10 minutes(Cheesbrough, 2006). The samples were stored in the Virology Laboratory of the Department of Microbiology ABU, Zaria at -15^0 analysed.

3.10 Screening of Samples

All samples were screened for HBsAg and HIV according to the manufacturers' protocols using rapid test immunochromatographic kits. Positive samples for HBV were confirmed using Enzyme Linked Immunosobent Assay (ELISA) kits.

3.10.1 Detection of HBsAg

The BioApex one Step HBsAg test kit manufactured by Richmond Hill, Ontario, Canada was used. It is a rapid immunochromatographic *in vitro* assay for the qualitative detection of HBsAg in serum.

3.10.1.1 Principles of HBV Rapid Test Strips

BioApex One Step HBsAg test is an antibody sandwich immunoassay. Colloidal gold conjugated monoclonal antibody reactive to HBsAg is dry-immobilized onto a

nitrocellulose membrane strip. When the sample is added, it migrates by capillary diffusion through the strip, rehydrating the gold conjugate. If present, HBsAg will bind with the gold conjugated antibody to form particles. These particles will continue to migrate along the strip until they reach the Test Zone (T) where they are captured by anti-HBsAg antibody immobilized there and a visible red line appears. If there is no HBsAg in the sample, no red line will appear in the T zone. The gold conjugate will continueto migrate alone until it is captured in the Control Zone (C) by immobilized goat, anti-mouse IgG antibody aggregating a red line.To serve as an internal process control, a control band should always be seen after test is completed. Absence of a colouredcontrol line in the control region is an indication of an invalid result.

3.10.1.2Test Procedure

All the samples for HBV were brought to room temperature (18-25^{0}C). The test device was removed from the foil pouch and labelled with the sample identifier. Fifty microliters of serum placed on the specimen pad on one end of the strip and was laid flat on a clean, dry and non-absorbent surface.A waiting time of 10 to 15 minutes was observed before the results were read.

3.10.1.3 Interpretation of Results

A pink coloured line was produced in the strip. The appearance of two distinct coloured lines, one in the control (C) region of the strip and the other in the test (T) region, indicated a positive result. The appearance of one coloured line in the control region only indicated a negative result. However, the appearance of non-visible band at the control region wasconsidered invalid results and was discarded.

3.10.2 ELISA

Hepatitis B surface Antigen ELISA Test is an enzyme-linked immunosorbent assay (ELISA) test designed for the qualitative detection of HBsAg in human serum or plasma.

3.10.2.1 Principle of HBV Test by ELISA

The HBsAg ELISA kit (manufacturer) utilizes Horseradish Peroxidase Enzyme (HRP) conjugated anti-human IgG and multiple recombinant HBsAg antigens to detect HBsAg antibodies qualitatively and selectively in serum or plasma. The test microwells are coated with multiple recombinant antigens including structure and non-structure proteins. The HBsAg antibodies, if present in the test sample, bind to the recombinant antigens immobilized on the microwell surface. The bound antibodies then react with HRPconjugated anti-human IgG, forming an antigen-HBsAg antibody-anti-antibody enzyme complex on the microwell surface. Upon the addition of Tetramethyl-benzidine (TMB) substrate, the HRP enzyme converts bound complex into a colour signal. The intensity of the colour is directly proportional to the concentration of HBsAg antibodies in the sample.

3.10.2.2ELISA Test Procedure HBV Seropositive Samples

All theHBV seropositive samples from the rapid test procedurewere brought to room temperature (18-25^{0}C) before use. The 25ml wash buffer provided was diluted (30x) with 29 portions of distilled water making quantity of 750ml and mixed well.A96 well microplate was used, the wells were then labelled (one reagent blank well, two negative control wells, two positive control wells and sample wells). Fifty micro litters of negative control, positive control and samples were transferred to the wells. In addition, 50µlHorseradish Peroxidase Enzyme (HRP) conjugate solution were dispensed into each well of the sample and control wells and mixed well. The plate was covered and

incubated for60minutes at 37^0C. The plate was then washed 5 times with the wash buffer after aspirating the content of the wells into a sink and blot dried on an absorbent paper. Exactly 100µl substrate Tetramethyl-benzidine was then added to each wells and incubated for 10minutes at room temperature. One Hundred microlitersof the stop solution was then added to each well, and the plate shaken gently. The Optical Density (O.D) of each well was read by setting the microplate reader wavelength at 450nm. (Hepatitis B surface Antigen Visual Test Kit -Catalog Number: 4029).

3.10.2.3 Interpretation of the ELISA results

The Optical Density ratio for each specimen was calculated by dividing the specimen OD value by the negative OD value (0.050). An OD ratio less than 2.10 was interpreted as negative, but when greater than or equal to 2.10 it was interpreted as a positive result.The negative result indicates that there is no detectable HBsAg in the specimen while positive result revealed that the patient might have been infected by HBV.

3.10.3 Detection of other Serological markers of HBV infection

All samples that were reactive to HBsAg test were further screened to detect other markers of HBV infection. These include hepatitis B surface antibody (anti-HBs), hepatitis B envelope antibody (anti-HBe), Hepatitis B envelope antigen (HBeAg) and Hepatitis B core antibody (anti-HBc). Diagnostic kit for HBV infection markerwere used for the qualitative detection of their presence.

3.10.3.1 Principles of HBV test for Marker

The diagnostic kit for HBV infection marker uses the colloidal gold and membrane chromatography technology and measures HBsAg, HBeAg in serum with dual-antibody sandwich method and measures HBsAb with dual-antigen sandwich method and measures HBeAb and HBcAb with neutralization competitive inhibition method.

3.10.3.2 Test Procedure and Interpretation

The test kit was removed from the pouch and labelled with the specimen identifier. The samples were brought to room temperature (20-30^{0}C). Two drops of serum was placed in each of the 5 sample wells and was read after 15minutes.

For the detection of HBsAb and HBeAb, the test strip has two distinct coloured zones known as the Test zone (T) and the Control zone (C). The appearance of two distinct purple bars,one in the control (C) region of the strip and the other in the test (T) region, indicated a positive result for HBsAg, HBsAb and HBeAg. However, appearance of one coloured line in the control region indicated a negative result and considered invalid when the purple bands was not detected in both control and test zones. While for the detection of HBeAb and HBcAb it was considered positive when there was only one purple bar in the control zone and negative when there were two purple bars in the Control(C) and the Test (T) zones and invalid when the purple bands was not detected both in control and test zone.

3.10.4Detection of HIV in Sera

For the detection of HIV antibodies in blood samples, a World Health Organization (WHO) approved kit (Determine HIV 1and 2 rapid test strips; Abbott laboratory- USA) was used. It is an immunochromatographic test for the qualitative detection of antibodies to HIV 1/2.

3.10.4.1Principles of the Test

A Sample is added to the sample pad and as it 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 antigens and synthetic peptides at the patient's window site. If antibody to HIV 1and 2 is present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line there.

If antibodies to HIV1 and 2 are absent, the antigen-selenium colloid flows past the patients' window and no red line are formed at that window site.

3.10.4.2 Test procedure for HIV

The protective foil cover of the test kit was removed and the kit labeled with the specimen identifier. Fifty microlitres of the test serum was added to the sample pad and the result was read visually after 15 minutes.

3.10.5 Interpretation of HIV Results

When two red bars appeared in both control and patient windows of the strip, the test was interpreted as positive. However, the appearance of only one red bar in the control window of the strip and the absence of a red bar in the patient window was interpreted as a negative test. When there was no red bar in the patient's window of the strip, the test was invalid and the result discarded.

3.11 Data analysis

The data obtained from the questionnaire and the result of the laboratory tests were analyzed using SPSS 17 (Statistical Package for Social Sciences version 17) and the results obtained were presented in tables, figures and graphs. The prevalence of each viral infection (HBV and HIV) and the co-infection was determined from the proportion of seropositive individuals in the total population under consideration and expressed as a percentage. The Pearson chi-square test was employed to determine the relationships between the demographic data and clinical information with HIV and HBV infection. P value of ≤ 0.05 was considered significant at 95% confidence interval.

CHAPTER FOUR

4.0 RESULTS

Of the 600 students that participated in the study, the prevalence of HBV was 9.2% (55/600) while HIV had a prevalence of 2.8% (17/600) (Table 4.1). One of the 600 students had co-infection of HBV and HIV giving a prevalence rate of 0.2% (1/600).

The characteristics of the study population are summarized in Table 4.2. There was a marked difference in the distribution of HBV and HIV by age group. Individuals who were above 40 years had the highest prevalence (30%: 3/10) of HBsAg while no virus was detected in the 36-40 years age group(0%: 0/20). However, there was a significant difference (χ^2 = 13.913, df=5, p=0.016) observed between age group and infection with the virus. For HIV infection, those aged 16-20 years had the highest seroprevalence (4.3%:5/115) while there was no infection reported for age groups 36-40 and those above 40 years (0 %: 0/20).

The distribution of HBV infection according to gender was significantly associated(χ^2 = 3.198, df=1, p=0.049; OR = 1.691; 95%CI= 0.946-3.022 36). However, higher

prevalence was recorded among the males students(11.1%: 36/324) than females students (6.9%:19/276). For HIV, it did not show any statistical difference (χ^2 = 0.339, df=1, p=0.367; OR = 0.751; 95%CI = 0.286-1.974). The highest prevalence was recorded among females students (3.3%:9/276) than in male students (2.5%: 8/324).

There was no observed statistical significant difference between the seroprevalence of HBsAg and HIV (χ^2 = 0.560, df=3, p=0.905) and (χ^2 = 2.109, df=3, p=0.550) and marital status. However, for both HBsAg and HIV the highest prevalence was recorded among

Table 4.1: Distribution of HBsAg and HIV and co-infection among student in ABU, Zaria.

Viral antigen	N0. Screened	No. positive (%)	No. negative (%)
HBsAg	600	55 (9.2)	545 (90.8)
HIV	600	17 (2.8)	583 (97.2)
HBsAg and HIV	600	1 (0.2)	599 (99.8)

Table 4.2: Distribution of HBsAg and HIV infection by demographic factors among students of ABU Zaria.

Demographic Factor	No.of samples examined	No. Positive *HBsAg(%)	No. Positive **HIV (%)	P value ≤ 0.05	Odds Ratio
Age (years)					
16 – 20	115	16(13.9)	5(4.3)		
21 – 25	253	24(9.5)	6(2.4)	^{H}p=0.016	
26 – 30	156	11(7.1)	5(3.2)	^{HI}p=0.824	
31 – 35	46	1(2.2)	1(2.2)		
36 – 40	20	0(0.0)	0(0.0)		
above 40	10	3(30.0)	0(0.0)		
Gender					
Male	324	36(11.1)	8(2.5)	^{H}p=0.049	^{H}1.691
Female	276	19(6.9)	9(3.3)	^{HI}p=0.367	^{HI}0.751
Marital Status					
Married	116	9(7.8)	1(0.9)	^{H}p=0.905	
Single	482	46(9.5)	16(3.3)	^{HI}p=0.550	
Widowed	1	0(0.0)	0(0.0)		
Divorced	1	0(0.0)	0(0.0)		
Family type					
Monogamy	85	4(4.7)	1(1.2)	^{H}p=0.056	^{H}0.257
Polygamy	31	5(16.1)	0(0.0)	^{HI}p=0.733	^{HI}1.369

Key:

H: Hepatitis B virus

HI: Human immunodeficiency virus

students who were single (9.5%:46/482 and 3.3%:16/482 respectively) while none of thewidowedordivorced student was infected (Table 4.2).

The distribution of HBsAg in relation to family type for married students was not significant(χ^2 = 4.142, df=1, p=0.056; OR = 0.257; 95%CI= 0.064-1.028). The highest prevalence was recorded among students who were in polygamous marriages (16.1%:5/31) as against students in monogamous relationships (4.7%:4/85). For HIV, the prevalence among those in monogamous marriages was higher (1.2%:1/85) as none of the students in polygamous marriages was infected (χ^2 = 0.368, df=1, p=0.733; OR = 1.369; 95% CI= 1.225-1.530).

The result was also analyzed according to other possible risk factors that might be associated with HBV and HIV, and the result was presented in (Table 4.3). There was no significant association between HBsAg prevalence and injection by uncertified medical personnel (χ^2 = 0.859, df=1, p=0.250; OR=0.859; 95%CI= 0.213-1.749). The viral Infection was higher in those who did not receive injection from uncertified medical personnel (9.6%: 51/534) and lower among students who receive injection from quacks (6.1%:4/66). Similarly, for HIV, there was no observed statistical significance (χ^2 =

0.011, df=1, p=0.575; OR=1.083; 95% CI =0.242-4.846). However, the prevalence was higher in those who received injection from uncertified medical personnel (3.0%:2/66) than those who had not received injection from uncertified medical personnel (2.8%:15/534).

Prevalence of HBsAg was higher among students who shared clothes with others (11.1%: 5/45) than in those who did not (9.0%: 50/555). However, there was no statistically significant difference in the prevalence obtained (χ^2 = 0.221, df=1, p=0.397; OR=1.083; 95% CI=0.477-3.344). Although for HIV there was no observed statistical difference (χ^2

Table 4.3: Prevalence of HBV and HIV infection among ABU Zaria students, in relation to some risk factors

Possiblerisk factor	No. of examined	No. Positive		P value	Odds ratio
		HBsAg(%)	HIV (%)	≤ 0.05	
Injection by uncertified medical personnel					
Yes	66	4(6.1)	2(3.0)	[H]p=0.250	*0.611
No	534	51(9.6)	15(2.8)	[HI]p=0.575	**1.083
Sharing of clothes					
Yes	45	5(11.1)	1(2.2)	[H]p=0.066	*1.263
No	555	50(9.0)	16(2.9)	[HI]p=0.631	**0.766
Sharing of bed space					
Yes	295	31(10.5)	8(2.7)	[H]0.164	*0.786
No	305	24(7.9)	9(2.9)	[HI]0.860	**0.917
Sharing of sharp unsterilized objects					
Yes	488	45(9.2)	14(2.9)	[H]0.546	*1.036
No	112	10(8.9)	3(2.7)	[HI]0.605	**1.073
Multiple sexual partners					
Yes	13	1(7.7)	0(0.0)	[H]0.662	*0.534
No	587	54(9.2)	17(2.9)	[HI]0.534	**1.023
Condom use					
Yes	138	11(7.9)	1(0.7)	[H]p=0.358	*0.823
No	462	44(9.5)	16(3.5)	[HI]p=0.69	**0.203

Blood transfusion					
Yes	7	1(14.3)	0(0.0)	[H]p=0.92	*0.982
No	593	54(9.1)	17(2.9)	[HI]p=0.817	**1.012
History of Infection in family					
Yes	42	11(26.2)	-	[H]p=0.000	
No	471	37(7.9)	-	-	
Don't Know	87	7(8.1)	-		
Engagement in menial jobs/petty trading					
Civil Servant	29	3(10.3)	0(0.0)	[H]p=0.298	
Student	552	48(8.7)	15(2.3)	[HI]p=0.001	
Farmer	11	2(18.2)	0(0.0)		
Other engagement	8	2(25.0)	2(25.0)		

= 0.066, df=1, p=0.631; OR=0.766; 95% CI= 0.99-5.909). The prevalence was higher among those that did not share clothes (2.9%:16/555) than those that did (2.2%:1/45).

Students who shared bed space had higher prevalence of HBV infection (10.5%: 31/295) than those who did not (7.9%: 24/305). Although, there was no significant association between the infection and sharing of bed space (χ^2 = 1.255, df=1, p=0.164; OR=1.375; 95%; CI=0.786-2.404). For HIV, students who did not share bed space had higher prevalence of the infection (2.9%:9/305) than those who shared bed space (2.7%:8/295). There was no statistically significant associationbetween sharing of bed space and the infection (χ^2 = 0.031, df=1, p=0.860; OR=0.917; 95% CI= 0.349-2.409).

Hepatitis B Antigen was more prevalent among those who had a history of Sharing Cups/Cutlery(9.2%: 45/488) than those who did not (8.9%: 10/112) although there was no significant association in the prevalence (χ^2 = 0.009, df=1, p=0.546; OR=1.036; 95%CI=0.505-2.125). Similarly, HIV infection was higher in those who had history of

Sharing Cups/Cutlery(2.9%: 14/488) than those who had not (2.7%:3/112). There is no significant difference(χ^2 = 0.012, df=1, p=0.605; OR=1.073; 95% CI=0.303-3.799).

There was no significant association between infection with HBV and history of multiple sexual partners (χ^2 = 0.035, df=1, p=0.662; OR=0.823; 95%CI=0.105-6.448). Prevalence was however higher in those that did not have of multiple sexual partners (9.2%: 54/587) than those that had (7.7%: 1/13). For HIV, there was no observed statistical significance (χ^2 = 0.387, df=1, p=0.534; OR=1.023; 95% CI=1.010-1.035). Higher prevalence was observed among those that had no history of multiple sex partners compared to those with a history (2.8%:17/587).

Hepatitis B surface antigen was detected with a higher prevalence in those that did not use condom (9.5%: 44/462) than those who used condom (7.9%: 11/138) although the difference was not significant (χ^2 = 0.308, df=1 p=0.358; OR=0.823; 95%; CI=0.413-1.640). For HIV, there was no statistically significant association relation between infection and the use of condom. However, a higher prevalence of infection was obtained in students that did not use condom (3.5%:16/462) than in those that used it (0.7%:1/138) (OR=0.203; 95% CI=0.27-1.548).

There was no observed statistically significant association between history of blood transfusion and HBsAg prevalence (χ^2 = 9.926 df=1 p=0.92; OR= 0.982; 95%CI=0.947-1.018). There was a higher prevalence among students who had been transfused (14.3%:1/7) as against those who had never been transfused (9.1%:54/593). Similarly, there was no observed statistical significant difference for HIV infection (χ^2 = 0.207 df=1

p=0.817; OR=1.012; 95%CI=1.003-1.021), between the students with no history of blood transfusion (2.8%:17/600) and those who had hadblood transfusion (0.0%:0/600)

In relation to family history, higher prevalence of HBsAg was observed among students who had history of infection (26.2%: 11/42) in their family compared to those who did not (7.9%:37/471). There was observed significant association in family history and HBsAg (χ^2 = 15.722, df=2, p=0.00)

The result was further analyzed according to involvement of the students in menial jobs/petty trading or not. Although, there was no statistical significant association between HBV detection and involvement in petty trading or not (χ^2 = 3.678, df=3, p=0.298). Students who engaged themselves in other businesses had the highest seroprevalence of HBV (25.0%:2/8), compared to those who were not involved (8.7%: 48/552). For HIV the difference in prevalence was statistically significant (χ^2= 15.472, df=3, p=0.001). However, the highest prevalence was observed among students engaged in other businesses (25.0%:2/8).

The result was further analysed based on students' knowledge and sources of information about the existent of HBV(Table 4.4). Two hundred and eighty six (286) of the students enrolled in the study indicated having knowledge of the existent of the infection while314 was ignorant about it. However, the result showed no significant association (χ^2= 1.394, df=1, p=0.314). Students who were totally ignorant about its existent had higher prevalence (9.9%: 31/314) compared to those who had knowledge about it (8.4%: 24/286; OR=0.836; 95% CI=0.478-1.462). Similarly, there was no statistically significant difference (χ^2= 6.989, df=4, p=0.136) between the students' sources of information and the presence of the infection. However, the highest prevalence was observed among

students who heard about the viral infection in the hospital (15.0%:9/60) and the lowest wasamong students who heard about it during seminars (3.2%:2/62).

Table 4.5 shows the distribution of HBV markers among the HBsAg reactive students. None of the HBsAg positive students had developed the Hepatitis B surface antibody (Anti-HBs), 7.3% (4/55) had Hepatitis B envelop antigen (HBeAg), 36.4% (20/55) had developed the envelop antibody (Anti-HBe) and 94.5% (52/55) had developed hepatitis B core antibody (Anti-HBc).

Table 4:4: Distribution of HBsAg among students according to Knowledge and sources of information about the HBV.

Variable	Total	No.positive HBsAg(%)	No. negative HBsAg(%)	P value ≤ 0.05	Odds Ratio
Knowledge of HBV					
Yes	286	24(8.4)283(90.1)		*0.314	*0.836
No	314	31(9.9)545(181.7)			
Sources of information					
Media	30	1(3.3) 29(96.7)		*p=0.136	
Seminar	62	2(3.2)60(96.8)			
Hospital	60	9(15.0)51(85.0)			
Others	46	3(6.5)43(93.5)			
No response	88	40(10.0)362(90.0)			

Table 4.5: Distribution of HBV markers in HBsAg reactive student in ABU, Zaria.

	N = 55	
Serological Marker	**No. Positive (%)**	**No. Negative (%)**
Anti- HBs	0(0.0)	55(100)
HBeAg	4 (7.3)	51(92.7)
Anti-HBe	20 (36.4)	35(63.6)
Anti-HBc	52(94.5)	3(5.5)

KEY:

Anti-HBs – Hepatitis B surface antibody

HBeAg – Hepatitis B envelop antigen

Anti-HBe – Hepatitis B envelop antibody

Anti-HBc – Hepatitis B core antibody

CHAPTER FIVE

5.0 DISCUSSION

This study was conducted to determine theseroprevalence of HBV and HIV infection and their co-infection among students of ABU Zaria. Results obtained from this study showed that 9.2% of the students were seropositive for HBsAg (a marker of HBV infection)and 2.8% were seropositive for HIV, while one student was infected with both viruses.

The seroprevalence of HBsAg of 9.2% reported in the present study is regarded as high seroprevalence level of HBV infection as per the WHO classification of assessing severity of HBV infection in HBV endemic countries. WHO defines low prevalence to be <2%, moderate prevalence as 2-8%, and high prevalence as >8% HBsAg positivity (WHO, 2010). Thesestudents werepotentially infectious (WHO, 2012).

This seroprevalence rate is lower than the 12.5% earlier reported amongst asymptomatic students in Ahmadu Bello University, Zaria(Main Campus)(Aminu, *et al.,* 2013),11.5% among Students of Nasarawa State University, Keffi (Pennap *et al.,* 2011) and 15.5% found among Medical students of Usman Danfodio University, Sokoto, (Alo *et al.,* 2013). Also, 11.0% was reported among Makerere University Medical Student (Pido and Kagimu, 2005).In contrast, Ugwuja and Ugwu (2009) reporteda lower seroprevalence of 4.1% among adolescents in Abakiliki, South Eastern, Nigeria and 4.7% among students in University of Uyo (Mboto and Edet, 2012).

The 2.8% seroprevalence obtained for HIV infection may be considered high compared to1.24% found at a Tertiary Health Care Center, Delta(Roche *et al.,* 2012*rem*) and 0.0% found among students in Tehran University of Medical Science students (Badie*et al.,* 2013). However, this rate is lower than the 9.9% found among students of tertiary institution in Malete-Ilorin Kwara state, Nigeria (Abubakar, 2012), 19.49% found among adults in Pankshin, Plateau state(Mawak *et. al.,* 2012) and 3.8% found among blood donors at Gondor University Teaching Hospital, North-west Ethiopia (Tessema*et. al.,* 2010).

Furthermore, only one student (0.2%) was found to be co-infected with the viruses. The study is similar tothe 0.5% among pregnant women, attending antenatal care in Kaduna,metropolis (Aba *et.al.,* 2012) and 0.77% prevalence among blood donors in Cameroon (Ymele*et. al.,* 2012). However, this value is low compared to the 3.9% co-infection obtained from a study amongpatients in a Nigerian Tertiary Hospital (Balogun*et al.,* 2012) and 4.3% reported among blood donors in selected Hospitals in

Zaria (Otori*et al.*, 2012). The reason for these variations may be related to the fact that infections tend to vary from one locality to another and from one country to another depending on the level of associated risk factors.

Higher seroprevalence of HBsAg was seen among studentsaged above 40 years, showing that the virus significantly associated with increasing age. The result is similar to the findings of Okonko *et.al.* (2012)who found higher prevalence in those above 40 years. This may be because older people might have obtained the infection in their younger age. In addition, in sub-Saharan Africa, HBV acquisition occurs early in life (Burnett *et. al.*, 2002).However, the small number of participants in some of the age groups might have affected the clear picture of the age related prevalence of HBV infection obtained. It is also very possible that other than HIV, many people may not be aware of other sexually transmitted viral infections and so continue to have unprotected sex with fellow HIV negative partners who might be chronic carriers of HBV. It has been noted that in Africa more than half of the population become HBV infected during their life time and about 8% of the inhabitants become chronic carriers (Pennap*et. al.*, 2011).

For HIV infection higher prevalence of thevirus was seen in age group 16-20 years, however, there was no significant difference between the infection and age.The result is similar to the findings of Ibadin and Enabulele (2008) in which a higher prevalence was obtained among age group 25-29 years and 14.9% among children in Abeokuta, Nigeria between age group 6-15 years, which is also not significant(Motayo *et. al.*, 2013) This result may be associated with higher sexual activities within these age groups. In all

epidemiological studies, younger age has always proved to be the most important factor because of their usual high desire for unprotected sex and higher promiscuous life.

In this study, HBsAg was significantly associated with gender. Prevalence was higher in males than females. This finding agrees with that of Koussi *et. al.* (2011) and Pennap *et al.* (2011) which showed higher prevalence of HBsAg among males than females. However it contrasts the report of Mustapha and Jibrin (2004), in which prevalence of HBsAg was higher in females than males. The finding in this study may be explained by themale's preponderance. However, it might not be unconnected with the higher rate of promiscuity among males than females which had been reported for Nigerians. These are behaviours characterized by casual and indiscrimate sexual intercourse, often with many people (UNSN, 2001). For HIV, prevalence was higher in females than males; however, there was no significant difference. This finding agrees with that of Otori *et. al.* (2012) who found higher prevalence among females than malesamong blood donors in selected Hospitals in Kaduna. Similarly, Koussi *et al.* (2011), Sule *et al.* (2010), and Tessema (2010) reported high prevalence among females than male in Abidjan. (additional report to compare)

In contrast, Ibadin and Enabulele (2008) reported higher prevalence in males than females.The predominance in female studentsmight be due to increase in vulnerability to HIV infection as a result of biological, social and economic disadvantages related to their gender(Tessema*et al.*, 2010). In addition, data on sexual behaviors indicate that risky behaviours are very common in Nigeria (Aba *et al.*, 2012), where many females involve themselves inunprotected sexual contact with men who may be infected with HIV.In sub-Saharan Africa, as well as worldwide, femalepopulation usually remains a key factor in the epidemiology of HIV and AIDS because 50% of all adults with HIV

infection are women predominantly infected via heterosexual transmission.Furthermore, females are the most severely affected and this could be as a result of the anatomy of the female sex organs(Okonko *et al.*, 2012).

The viruses were not associated with marital status of the students although a higher prevalence was recorded among students who weresingle. This result is similar to the report of Okonko*et.al.* (2012)which showed no significant association between HIV and marital status. Similarly,Ejele*et. al.* (2004) reported that single/unmarried patients constituted the highest proportion of those with HIV/HBV co-infection.This may be explained by the fact that promiscuity and unprotected sexual behaviours among singles/unmarried might be higher than among the married therefore, increasing the risk of acquiring the viruses due to their inability to stick to only one sexual partner. However, the results contrastthat of (Otori *et al.*, (2012; Aminu*et. al.* (2013), where HBsAg and HIV infection were detected with higher prevalence among married students.

In relation to family type, HBsAg seropositivity was higher among those in polygamy compared to those in a monogamous relationship. This may be because of the multiple sexual relationships in polygamous families as well as large population and a higher probability of person-person contact in polygamous homes. Since, the virus can easily spread throughvarious body fluids, poor hygiene could easily encourage its transmissionamong the polygamous families. Human Immunodeficiency Viral infection based on the result of this study, was higheramong students in monogamous thanin polygamous marriages. This is contrary to the report by Otori *et al.* (2012), which

reported higher prevalence in patients in polygamous relationship. This could be as a result of differences in study population and the occurrence might be due to chance.

Infection with the viruses was not significantly associated with injection by quacks in this study.It iscontrary to report by Adekanle *et.al.* (2010) and Nwakediuko (2010) who found a significant association.A Higher prevalence of HBsAg was detected among students that had no history of injection by uncertified medical personnel. This supports the fact that it is becoming clearer now that many cases of HBV infection are known to result from less apparent modes of non-percutaneous or covert percutaneous transmission (Talwar and Arora, 2010cited in Pennap*et. al.,*2011). However, in developing countries, exposure to contaminated therapeutic injection equipment are common in many settings because of lack of awareness of infection control practices, lack of resources for sterilization and the purchase of new disposable equipment, and cultural preferences favouring overuse of injections. Contaminated injections caused an estimated 21 million HBV infections worldwide in 2000, accounting for 32% of all new infections (Hauri *et al.,* 2004).Nwakediuko (2010) reported that nearly half of the patients in Nigeria (48%)with various forms of HBV-related liver disease gave a history of having received injections from uncertified medical personnel. However, ahigher seroprevalence of HIV was detected among student that had history of injection by uncertified medical personnel. The use of unsafe injections due to the negligence of some medical practitioners and quacks has resulted to 5% of new HIV infections. It was also estimated that up to 160, 000 HIV and HBV each year are attributable to injection by quacks (Kermode, 2004).

Hepatitis B surface Antigen seropositivity was higher among students who shared clothes and bed space. While higher prevalence of HIV was detected among those that did not

share these. However, there was no observable statistical significant association between thesepotential risks factors and the infections. This finding agrees with Ndako *et al.* (2011), who found no significant association between these risks factors with the infection. Sharing of bed or clothes is a predominant lifestyle of students in this community; hence increasing the chances of acquiring especially the HBV infection. Close personal contact has been reported among students of Main Campus, ABU, Zaria, where more than six students share a room in the hostel (Aminu *et al.,* 2013).The implications of sharing of clothes if found associated with these viruses could be detrimental, especially HBV which could be found in saliva, sweat, tears, urine, breast milk and any body fluids(Elgouhari *et al.,* 2008). The virus can easily be transmitted through abrasion, wounds. It becomes highly transmissible when the person has developed HBeAg.

Hepatitis B Virus and HIV seroprevalence was higher among those who confessed to engage in sharing cups and cutleries. This finding is similar to Mboto and Edet (2012), who found higher prevalence of HBsAg among students who shared unsterilized sharp object in University of Uyo. In addition, Ndako *et. al.* (2011) reported, history of practicing high risk behaviours such as sharing of sharp objects and tooth-brush among students. However, the study was not significantly associated with the infection and this corresponds with the study of Dawaki and Kawo that these risk factors are poorly associated with the infection (Dawaki and Kawo, 2006). However, this is a practice that can easily expose one to blood or other body fluids which might leads to the acquiring of the viral infection.

Having multiple sexual partners was not significantly associated withHBsAg and HIV infection. Mboto and Edet (2012) reported that those with multiple sexual partners were significantly associated with HBsAg infection. Similarly, Comia and co-workers (1999) found out that, this factor was not significantly associated with the infection but only proves that this factor could equally be a risk for infection (Comia *et. al.,* 1999). According to this study,HBsAg and HIV were highest among students that did not have multiple sexual partners, similar to the findings ofAdekanle *et al.*(2010) who reported higher HBsAg prevalence in those without multiple sexual partners than those who had. The finding of this study is contrary to Pennap*et.al.* (2011) who reported higher prevalence of HBsAg among Students of a Nigerian Tertiary Institution with multiple sexual partners. Reasons why those with multiple sexual partners had lower prevalence could be students might have been careful and usually take necessary precaution when negotiating new sexual partners.In addition, those infected could have gotten the viral infectionby chanceor through vertical transmission, parentally or abrasion by body contacts.

This study found no significant difference in terms of condom use HBsAgand HIV seropositivity.However, there were higher HBsAg and HIV infection rates among students that did not use condoms when compared to those that used condoms. This finding is similar to the report by Otori*et al.* (2013), where the highest prevalence of HIV was reported among patients that did not use condoms prior to infection. This could be because when sex is unprotected, or a new condom is not properly used each time, the risk ofHBsAg and HIV increases. Data on sexual behaviors indicated that risky behaviors are very common in Nigeria; whilecondom 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 (Otori *et. al.*, 2013).

In relation to history of blood transfusion for both HBsAg and HIV, there was no observable significant association with viral infection. However, most of the students that were seropositivefor HBsAg hada history of blood transfusion. This may be due to the transfusion of improperly screened blood. Because not all Nigerian hospitals have the technology to effectively screen blood (especially for HBV markers). Therefore, there is a risk of using contaminated blood. For, HIV, a higher seroprevalence was observed in those that have not had ablood transfusion. This issimilar to the findings of Buseri*et al*. (2010).However, this result contrasts that of Otori*et al.* (2013), who reported a significant association between HIV and blood transfusion. This could agree with the fact that HIV transmission is mainlyconnected with the unprotected heterosexual relationship with an infected partner (Buseri *et. al*., 2010).In addition, few number of participants only indicatedhaving history of blood transfusion, this might have affected the clear picture of the HIVseroprevalence.

Higher prevalence of HBsAg was observed among students who had history of infection in their family compared to those who did not.However, there was observable statistical significant difference between family history of the students and HBV infection.This could be due to close contact usually observed among family members especially in Africa where sharing is a common characteristic and any person that tends to isolate himself from sharing may be considered as cultural disregard, social disregard or showing indifference or disunity, thereby, increasing the chances of contracting the viral infectionas a results ofexposure to contaminated blood or body fluids.

There was no association between HBsAg and the students' involvement in menial jobs/petty trading. However, the study was in contrast with Bwogi *et. al.* (2009) in which prevalence was significantly, associated with occupation of students. The infection was higher among students who usually engaged in menial jobs/petty trading. This is in agreement with the finding of Bwogi *et. al.* (2009) who found that being in a professional or service occupation was associated with lower risk of lifetime HBV infection in women compared to other occupational categories. Similarly, Ejele *et.al,* (2004) reported that commercial sex workers had the highest prevalence of HBsAg among the occupational groups. Based on this study, individuals can get HBsAg by chance. While for HIV, there was a significant association between HIV and students' engagement into menial jobs/petty trading. Similarly, infection was higher among students who usually engaged in other businesses. This showed that student's engagement into other menial jobs/petty trading had greater role to play in the distribution of HIV. Factors that were thought to lead to the increase of seroprevalence were low socio-economic status which might have lead the students to go extra mile, engaging into some risk behaviours in other to improve their standard of living on campus, which might have predisposed them to the virus. Furthermore, is usually true especially among some female's students who usually engaged as casual commercial sex workers.

Prevalence of HBsAg among students in relation to knowledge of the virus showed higher prevalence among students who were not aware of the virus and its mode of transmission. Over half of the students were not aware of the virus and its mode of transmission. This finding confirms that of Aminu*et al.* (2013), that reported that one third of the students in ABU, Zaria were not aware of hepatitis B and the mode of transmission. It has becomeclearer now that cases of HBV infection could be attributed to

less apparent knowledge about the virus as high endemicity was recorded in this findings. Furthermore, it has been noted that in Africa more than half of the population become HBV infected during their life time and about 8% of inhabitants become chronic carriers(Pennap, *et. al.,* 2011). Some of these students might indulge in behaviours that had been reported as possible risk factors for HBV transmission which they may not be aware of it. This is pathetic as this high prevalence is in a population that was thought to be educatedand apparently healthy and also falls within the group of prospective blood donors.

In regard to sources of information of HBV, the study found higher prevalence in those who obtained information about the virus inHospitals. However, majority of the students indicated seminars as their source of information.There was significant difference between the sources of information and the infection. This is similar to findings by Aminu*et. al. (*2013) who reported that sources of information or knowledge of the virus was mostly gotten from discussion with friends and from lectures.It could be that most of the students came to the Hospital to participate in the research work or those who came with certain illness which eventually gave consent to participate in the study, which led to the detection of HBV.Furthermore, is awell-known factthat most people especially in developing countries do not usually go for medical checkup to know their health status until when they are downwith certain illness that may request for general test to be perform. However, Infection with HBV could remain asymptomatic for decades until it begins to manifest later in life and the victim may continue to transmit thevirus to other people. In addition, these findings highlight the gaps that are important to address as lack of good information could probably be the course of the transmission of the viral

infection. However, 31% of the students did not indicate their sources of information about the HBV; it could be that they forgot.

The distribution of HBV markers in the HBsAg reactive students indicates that none of the students had developed anti-HBs. The presence of anti-HBs is generally interpreted as indicating recovery and immunity from hepatitis B virus infection. Anti-HBs also develop in a person who has been successfully vaccinated against hepatitis B. It could be that the students might have not been vaccinated even though they are at risk for their continual exposure. It is a common slogan among the African's people to say "No disease in Africa" therefore, subject themselves to some risk behaviours without any consideration of medical check-up which may pose great danger of acquiring the virus, since it can remain asymptomatic for decades.It means that all the students were having recent infection and have not yet recovered from natural infection (Shepard, *et al.*, 2006).

Furthermore, 4(7.3%) of the HBsAg reactive students had HBeAg.Its presence indicates that the virus is replicating and the infected person had high levels of HBV. The prevalence was higher compared to the study by Otegbayo*et al.* (2003), who reported 2.3% positive forHBeAg.It means four of the students were highly infectious.Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity. Therefore, the four students could potentially transmit the virus through contacts of body fluids or blood. The presence of HBeAg in the students could increase the chances of the spread of the infection to others. Since sharing is the major characteristic of the student of ABU main campus. In addition,there is a high risk of developing liver cirrhosis or hepatocellular carcinoma later in life as the virus keep on replicating.Most chronically infected

individuals present with HBeAg-positive ten to thirty years after their initial infection (Fattovich, 2003).

The study also reported36.4% of the individuals had developed the envelop antibody (anti-HBe). This is generally produced by the immune system temporarily during acute HBV infection;it indicates lower levels of HBV or resolution of infection. 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.It indicates that they students are not infectious and may not transmit the virus. It also shows the processes of recoveryfrom the infection.

In addition, 94.5% of the individuals had developed hepatitis B core antibody. Anti-HBc is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. IgG anti-HBc generally remains detectable for a lifetime (WHO, 2012). It is therefore difficult to determine the time point at which these students became infected with the HBV.

Summarily, HBeAg which is the representative of viremic stage occurs early in the incubation period, concurrently or shortly after the first appearance of HBsAg. High levels of IgM-specific anti-HBc are frequently detected at the onset of clinical illness. Antibody to HBsAg is first detected at a variable period after the disappearance of HBsAg. Before HBsAg disappears, HBeAg is replaced by anti-HBe, signaling the start of resolution of the disease. Anti-HBe levels often are no longer detectable after six months. Low titres of IgM anti-HBc are found in the sera of most chronic HBsAg carriers.

CHAPTER SIX

6.0 CONCLUSION

In conclusion, this study has found the seroprevalence of HBV infection among students in ABU, Zaria to be 9.2%, which implies that thevirus is highly endemic among the students based on WHO recommendation.Human Immunodeficiency Virus seroprevalence and HBV and HIV co-infection were observed to be very low(2.8% and 0.2% respectively).

Most of the risk factors studiedwere not significantly associated with the viral infections. However, family history and student's engagement in other menial jobs showed significant association with HBV and HIV respectively. This indicates that close contact among family members and economic disadvantages of some of the students mightbe a predisposing factor.

Hepatitis B virus Markers among the reactive students showed most of the studentswerehaving recent infection. While four were highly infectious, 20 were resolving while fifty two showed ongoing infection. The result indicates that few of the students can transmit the virus and most were not vaccinated against HBV infection and therefore can serve as potential reservoir for the transmission of the infection.

Furthermore,demographic factors studied, revealed that age and gender was significantly associated with HBV infection.

6.1 RECOMMENDATION

In view of the seroprevalence of HBVobtained in this study, it is recommended that:

I. Resources should be made available as a matter of urgency so as to limit further spread of the viral infections by screening and vaccination of students during the first year of study since HBV vaccine has been shown to be protective.
II. Overcrowding of rooms should be avoided as HBVin infection can spread faster due to close contact.
III. Screening and counselling services should be offered free of charge in all institutions of higher learning in the country for HIV and HBV.
IV. Sensitization lecture should be organized once in two months for students to be well informed.

6.2 LIMITATION OF STUDY

Lack of funds to increase the sample size to meet up with the large population.

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APPENDIX I: ETHICAL APPROVAL

MINISTRY OF HEALTH, KADUNA STATE

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

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

MOH/ADM/744/VOL.1/221 **15th March, 2013.**

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ETHICAL APPROVAL

I have been directed to convey the Ministry's approval to Isa Ibrahim, Department of Biological Sciences, ABU – Zaria, Kaduna State. Topic: **SERO-PREVALENCE OF HEPATITIS B VIRUS AND HUMAN IMMUNODEFFICIENCY VIRUS AMONG STUDENTS OF AHMADU BELLO UNIVERSITY, ZARIA - NIGERIA.**

It is expected that necessary assistance be accorded to him in the process of his research, please.

However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honorable Commissioner, Health as soon as it is concluded as you have the assurances of my highest regards.

Yours Faithfully,

F.A. KURAH (MRS)
SECRETARY, ETHICAL COMMITTEE

APPENDIX II: CONSENT FORM

I **IsaIbrahim** a Postgraduate Student of Biological Sciences Department inAhmadu Bello University, Zaria will be undertaking a research work on the topic: **Seroprevalence of Hepatitis B Virus and Human Immunodeficiency Virus among Students of Ahmadu Bello University, Zaria, Nigeria.**

The purpose of the research is to determine the prevalence of hepatitis B and Human Immunodeficiency viral co-infection and the risk factors among students of tertiary institution.

This study will provide information and markers for the diagnoses of the etiology for management of this kind of infection, baseline data for further researches, vaccine production and recommendations that will be helpful to the public on the prevention and control of the infection.

Your participation will involve 3mls of blood taken from you using a needle which will cause a little pain to you. I will appreciate your participation in this study.

Moreover, all information obtained will be treated as confidential and used only for the research purpose. Participation is voluntary. All investigation will be free. You can withdraw from the research exercise at any point in time.

Kindly fill the space below if you have given your consent to participate.

I ..... give my consent to participate in this study. I have been duly informed of the process and have given my free will and i am not under any pressure.

....

Signature/ thumbprint of subject Date

....

Signature of witness Date

....

Signature of the investigator Date

APPENDIX III: QUESTIONNAIRE

Seroprevalence of Hepatitis B Virus and Human Immunodeficiency Virus among Students of Ahmadu Bello University, Zaria, Nigeria.

This questionnaire is a part of research exercise; it will provide the necessary basic information needed for the research study for appropriate evaluation and conclusion. All information obtained will be treated as confidential and used only for the research purpose. Please correctly fill or tick answers where it is required. **Thank you.**

SECTION A: Bio data

1 Reference Number: …….

2 Age: ……..

3 Sex: Female( ) Male ( )

4 Marital status: Single( ) Married( ) Divorced( ) Widowed( )

5 Type of marriage: monogamy( ) Polygamy( )

6 Number of marriages: ……

7 State of origin ……..

8 State of residency: …….

9 Occupational : Civil servant( ) Self-employed( ) Farmer( ) Student( ) others ( )

SECTION C: Risk Factors

10. Do you have any knowledge on Hepatitis? Yes ( ) No ( )

If yes, how and where did you hear about it?.....

11 Any family history of hepatitis? Yes ( ) No ( ) Don't know ( )

If yes, what type of hepatitis? ……

12 Have you done Hepatitis test before? Yes ( ) No ( )

13. Have you ever receive Injection from Unqualified Medical Personnel and Parents? Yes ( ) No ( )

14 How many are you in a room……..

15 Do you share cloths with friends? yes( ) No( )

16 Do you share bed space? yes( ) No( )

17 Do you share cups and cutleries? yes( ) No( )

18 What is your HIV status? Positive ( ) Negative ( ) Don't know ( )

19 Have you been transfused before?Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Five times >Five times ( )

20 How many times have you received transplant?

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

21 Do you have multiple sexual partners? Yes ( ) No ( )

22 Do you use condom? Yes ( ) No ( )

23 If yes, how often? Always ( ) Not always ( )

APPENDIX IV: ELISA RESULT

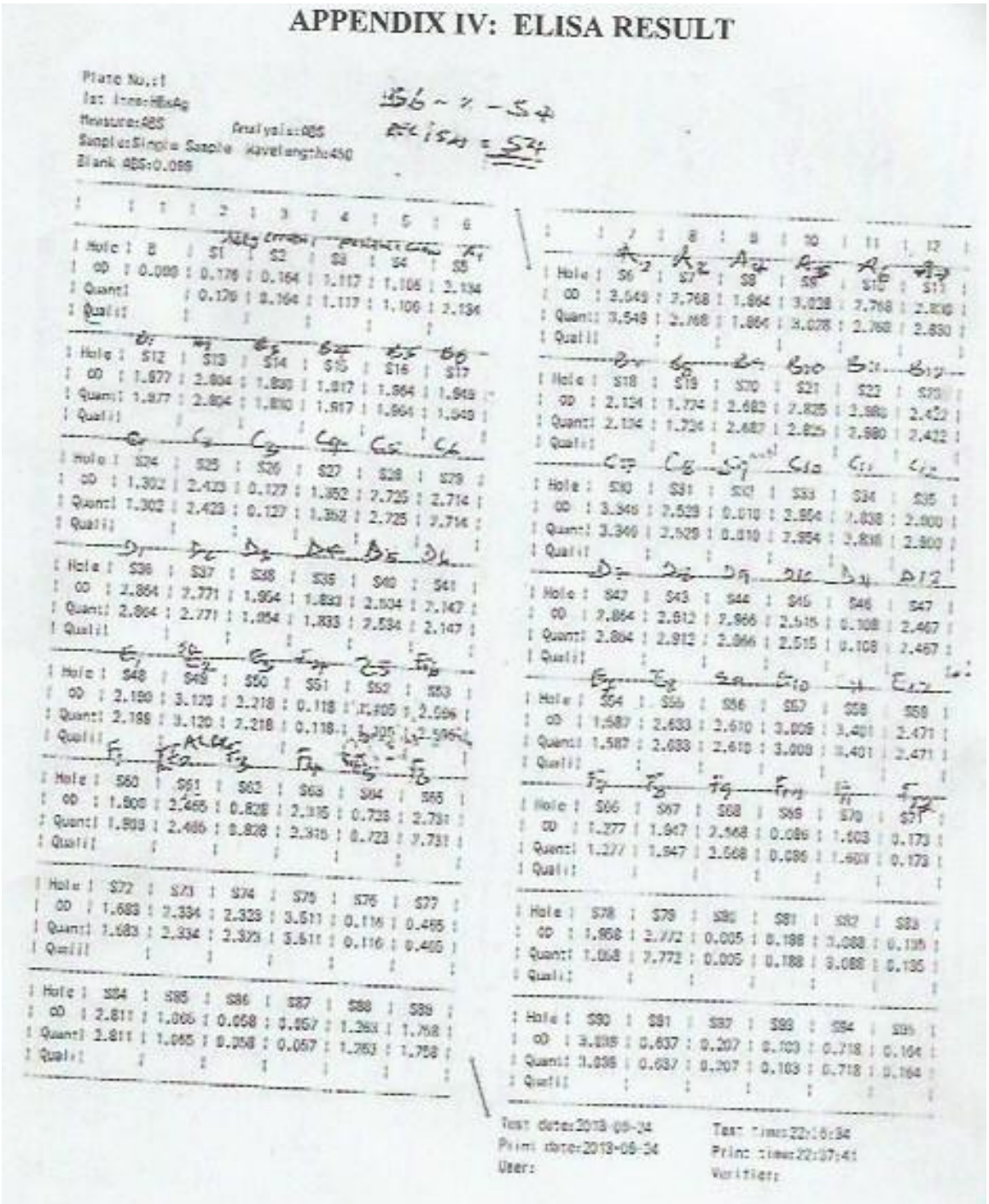


Plate No.:1
Test Item:HBsAg
Moisture:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.086

	1	2	3	4	5	6
Hole	B	S1	S2	S3	S4	S5
OD	0.088	0.176	0.164	1.117	1.106	2.134
Quant		0.176	0.164	1.117	1.106	2.134
Quali						

Hole	S12	S13	S14	S15	S16	S17
OD	1.877	2.804	1.810	1.817	1.864	1.949
Quant	1.877	2.804	1.810	1.817	1.864	1.949
Quali						

Hole	S24	S25	S26	S27	S28	S29
OD	1.302	2.423	0.127	1.362	2.725	2.714
Quant	1.302	2.423	0.127	1.362	2.725	2.714
Quali						

Hole	S36	S37	S38	S39	S40	S41
OD	2.864	2.771	1.964	1.833	2.534	2.147
Quant	2.864	2.771	1.964	1.833	2.534	2.147
Quali						

Hole	S48	S49	S50	S51	S52	S53
OD	2.199	3.120	2.218	0.118	1.805	2.096
Quant	2.199	3.120	2.218	0.118	1.805	2.096
Quali						

Hole	S60	S61	S62	S63	S64	S65
OD	1.900	2.465	0.828	2.315	0.723	2.731
Quant	1.900	2.465	0.828	2.315	0.723	2.731
Quali						

Hole	S72	S73	S74	S75	S76	S77
OD	1.683	2.334	2.323	3.511	0.116	0.465
Quant	1.683	2.334	2.323	3.511	0.116	0.460
Quali						

Hole	S84	S85	S86	S87	S88	S89
OD	2.811	1.065	0.058	0.057	1.263	1.758
Quant	2.811	1.065	0.058	0.057	1.263	1.758
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	2.549	2.768	1.864	3.028	2.768	2.830
Quant	2.549	2.768	1.864	3.028	2.768	2.830
Quali						

Hole	S18	S19	S20	S21	S22	S23
OD	2.124	1.774	2.682	2.825	2.980	2.422
Quant	2.124	1.774	2.682	2.825	2.980	2.422
Quali						

Hole	S30	S31	S32	S33	S34	S35
OD	3.349	2.529	0.010	2.954	2.838	2.800
Quant	3.349	2.529	0.010	2.954	2.838	2.800
Quali						

Hole	S42	S43	S44	S45	S46	S47
OD	2.864	2.812	2.866	2.515	0.108	2.467
Quant	2.864	2.812	2.866	2.515	0.108	2.467
Quali						

Hole	S54	S55	S56	S57	S58	S59
OD	1.587	2.633	2.610	3.009	3.401	2.471
Quant	1.587	2.633	2.610	3.009	3.401	2.471
Quali						

Hole	S66	S67	S68	S69	S70	S71
OD	1.277	1.947	2.568	0.086	1.603	0.173
Quant	1.277	1.947	2.568	0.086	1.603	0.173
Quali						

Hole	S78	S79	S80	S81	S82	S83
OD	1.868	2.772	0.005	0.188	3.088	0.135
Quant	1.868	2.772	0.005	0.188	3.088	0.135
Quali						

Hole	S90	S91	S92	S93	S94	S95
OD	3.836	0.637	0.207	0.103	0.718	0.164
Quant	3.836	0.637	0.207	0.103	0.718	0.164
Quali						

Test date:2013-09-24 Test time:22:16:34
Print date:2013-09-24 Print time:22:37:41
User: Verifier: