SERO-PREVALENCE AND IDENTIFICATION OF CIRCULATING GENOTYPES OF HEPATITIS B VIRUS AMONGST PATIENTS ATTENDING SOME HOSPITALS IN NIGER STATE

BY

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ABSTRACT

Hepatitis B virus (HBV) infection remains a major public health problem globally and accounts for about one million deaths worldwide in spite of the use of available vaccine and medications for mitigation of the disease. This study determined the seroprevalence, distribution of HBV infection, factors associated with the infection, and the genotypic patterns amongst patients attending selected hospitals in Niger State, Nigeria. A total of 500 blood samples were collected from five selected hospitals in Niger state. The samples were screened using Hepatitis B Surface Antigen (HBsAg) test kit for qualitative detection of Hepatitis B Surface Antigen in serum. DNAs were extracted from the 65 positive sera. HBV DNA genotyping and sequencing were performed on the HBsAg - positive samples, using Multiplex-PCR. For HBV genotyping, the second amplification products were analyzed using Agarose-Gel electrophoresis. Out of the 32 positive samples genotyped, five (5) were successfully sequenced and subjected to phylogenic analysis. Prevalence of Hepatitis B infection amongst the studied population was 13.0%. Female participants had a non-significantly higher prevalence (6.8%) of HBsAg infection than their male counterparts (6.2%, $p>0.05$). Participants within 41- 50 years age group recorded a significantly higher rate of infection (5.2%), compared to patients ≥ 50 years with 2.3% prevalence ($p>0.05$). The Civil Servants had a higher percentage prevalence of 6.4% followed by house wives (3.2%), while the least prevalence was observed among the students (1.6%). Patients without history of blood transfusion had significantly higher percentage prevalence (7.8%) compared to those with history of blood transfusion (5.2%, $p>0.05$). The results also revealed that participants with polygamous family type recorded higher prevalence of HBV infection (11.4%) compared to those belonging to the monogamous type (1.6%). Five (5) genotypes were identified amongst the 32 positive samples as follows: genotype A: 14 (34.1%); genotype C: 4 (9.8%); genotype D: 1 (2.4%); genotype E: 19 (46.3%); and genotype F: 3 (7.3%). Fifteen (15) samples accounting for 46.9% had co-infection of multiple genotypes; with A/E accounting for 31.25% (10/32), followed by C/ E, 9.375% (3/32) and the least was A/F, 3.125% (1/32). Most of the co-infections were a combination of genotype E, the predominant genotype and others. **S**equencing and phylogenic analysis of the five queried samples showed that genotype E had a percentage identity ranging from 98-100. The results obtained in this study shows that HBV is hyper-endemic in the study area and the circulation of the five HBV genotypes (A, C, D, E and F) amongst the study population of Niger state was confirmed; with HBV genotype E (HBV-E) predominating amongst the study population, probably due to the in ability of the innate immune response of the host to resist the strain. There is the need for more awareness and sensitization of people on the prevalence of HBV infection in Niger State. People need to be enlightened on the modes of transmission and spread of the virus through different information media outfits.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Hepatitis is a condition defined by the inflammation of the liver (Thio *et al.*, 2002). It is caused by infectious and noninfectious agents. The former includes hepatotrophic viruses (hepatitis A, B, C, D and E) of which hepatitis B is the most infectious and most virulent, while the latter is induced by an over active immune system, use of drugs, alcoholism, chemicals and environmental toxins (Thio *et al.*, 2002).

Hepatitis B virus (HBV) is a member of the Hepadnavirus family; it is a partially double-stranded DNA virus with a core antigen surrounded by a shell containing hepatitis B surface antigen (HBsAg) (Hollinger and Dienstag, 1990; Thio *et al*., 2002). HBV has numerous antigenic components such as hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) (Chisari *et al*., 1989). The HBV genome harbors 3200 nucleotides and it can encode four groups of proteins and their regulatory components by shifting the reading frames over the same genetic matter (Chisari *et al*., 1989).

Hepatitis B virus (HBV) is a public health problem and it is responsible for 60-80% of liver cancer worldwide despite the infection being preventable through vaccination (WHO, 2014). The initial infection after exposure is acute (resolved within six months), with either complete recovery, or progression to chronic disease that lasts more than six months (WHO, 2014).

The risk of chronic infection is related to age, approximately 90% of infected infants become chronically infected, compared with 2%–6% of adults (WHO, 2014).

Transmission of this disease is commonly through blood transfusion, blood products, body fluids (Urine, semen, sweat, saliva and tears), use of contaminated needles and mother-to-child transmission through infected birth canal (Brooks *et al*., 2007). Approximately 70–140 million of the chronic HBV infections and 250,000 of the 1.3 million HBV-related deaths recorded each year around the world occur in Africa (Hubschen *et al.,* 2008).

There have been previous studies on sero-prevalence of HBV amongst some target groups like pregnant women attending ante-natal care clinics (Thio *et al*., 2002; Kuta *et al*., 2014; Adabara *et al*., 2012, Olayinka *et al*., 2016; Berinyuy *et al*., 2019) with different percentage prevalence reported. The present study was designed to determine sero-prevalence of HBV taking into consideration the following risk factors: sharing of utensils, gender, marital status, blood transfusion, family type, age, and occupation; attending different hospitals located in three zones of Niger state, Nigeria. This is the first study designed to cover this scope.

1.2 Statement of the Research Problem

Hepatitis B virus (HBV) is a public health challenge worldwide (WHO, 2017). Many people are harboring the virus unknowingly due to reluctance of people to undergo routine screening. Lack of awareness by the larger population on some remote modes of transmission of the disease such as the use of contaminated utensils, needles, clippers, shavers, mother to child transmission through infected birth canal and contaminated body fluids (urine, sweat, semen, saliva and tears) (Brooks *et al.,* 2007).

The prevalence of hepatitis B virus infection in sub Saharan Africa, including Nigeria has been reported to be high (WHO, 2017). About 75% of its population likely to have been exposed to the virus at one time or the other in their lives. HBV may persist in the

liver for lifetime (Jia and Zhuang, 2007). Which not only causes severe HBV related sequalae such as cirrhosis and hepatocellular carcinoma but also constitutes the reservoir of the virus (Zou *et al*., 2001).

Prevalence rate of 4.3 % was reported from Port Harcourt (Akani *at al.,* 2005). 5.7% from Ilorin (Agbede *et al.,* 2007). 11.6% from Maiduguri (Harry *et al.,* 1994). 8.3% from Zaria (Jatau and Yabaya, 2009). 23.3% from patients attending all clinics at the Aminu Kano Teaching Hospital (Nwokedi *et al.,* 2006). Adabara *et al.* (2012) reported 6.5% Prevalence of Hepatitis B Virus among Women Attending Antenatal Clinic in the General Hospital, Minna, Niger State.

In Niger State, the current picture of the circulating genotypes of the virus has not been established. Therefore, this study is an attempt to identify the circulating genotypes of the virus in the study areas.

1.3 Justification for the Study

The need for continuous surveillance and monitoring of the prevalence of HBV infection in endemic regions cannot be over-emphasized. About 18 million Nigerians were infected with HBV (Olayinka *et al.,* 2016). Expectedly, the number keeps growing largely because of the chronic nature of the infection. There is a gap of study on current molecular characteristics of the HBV in Niger state, Nigeria. The present study was therefore designed to provide information on HBV sero-prevalence, and identification of the circulating HBV strains in Niger State.

Such information would assist in planning and strategizing the management of this fatal health challenge. It is envisaged that information obtained from the strains isolated will form the basis for future development of target specific Hepatitis B virus vaccines.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study was to determine the Sero-prevalence and Identification of circulating genotypes of Hepatitis B Virus (HBV) from patients attending some Hospitals in Niger State.

1.4.2 Objectives

The objectives of this study are to:

i. determine the prevalence of HBsAg infection amongst patients attending some hospitals in Niger State.

ii. identify the socioeconomic/risk factors associated with the rate of HBV infection.

iii. identify the circulating genotypes of hepatitis B virus amongst patients attending some hospitals in the study area.

iv. examine the degree of divergence of HBV genotypes from the different study sites using phylogenic analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hepatitis B Virus

The hepatitis B virus (HBV) is the prototype member of a family of hepatotropic DNA viruses, the *Hepadnaviridae,* that replicate by reverse transcription of an RNA pregenome. HBV primarily infects humans, although chimpanzees, Chacma baboons, and tree shrews are also susceptible to infection (Hu *et al*., 2000; Cao *et al*., 2003). Other hepadnaviruses infect mammals (orthohepadnaviruses) or birds (avihepadnaviruses) (Schaefer, 2007).

HBV comprises eight genotypes (A to H) with distinct virological characteristics and geographic distribution (Kramvis *et.al.*, 2005 and Schaefer, 2007). Each genotype differs from the others by more than 8% of its nucleotide sequence. Genotypes may influence the disease caused, although further analysis of this association is required (Schaefer, 2007). The variability of the HBV genome may be further increased by recombination among genotypes, especially B/C and A/D.

HBV genotypes, with the exception of genotypes E and G, are divisible into sub genotypes. Each sub genotype differs from each other's by more than 4% of its nucleotide sequence. The number of sub genotypes per genotype described to date ranges from three to five (Kramvis *et al.*, 2005 and Schaefer, 2007).

Hepatitis B virus (HBV) is a member of the hepadnavirus family (Zukerman and Baron 2009). The virus particle consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30–42 nm in diameter. The

nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Lorcanini, 2004).

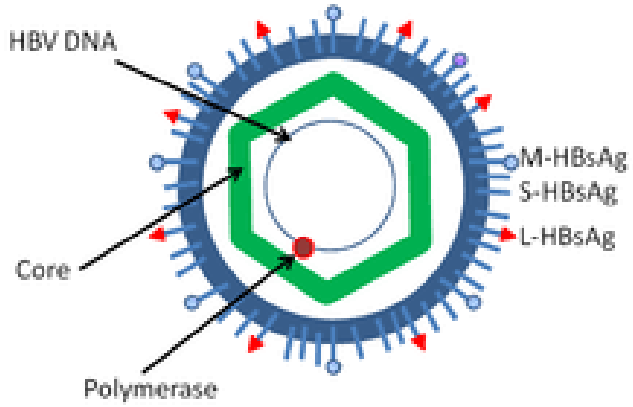

Figure 2.1: The structure of Hepatitis B virus

Source: (CDC, 2015)

The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses. The 42 nm virions, which are capable of infecting liver cells known as hepatocytes, are referred to as "Dane particles". (Harrison, 2009). In addition to the Dane particles, filamentous and spherical bodies lacking a core can be found in the serum of infected individuals. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigens (HBsAg) and is produced in excess during the life cycle of the virus. (Harrison, 2009). HBV is an enveloped virus, measuring 42–47 nm in diameter, with an icosahedral nucleocapsid that encloses a partially double-stranded relaxed-circular (rc) DNA genome covalently bound to the viral polymerase.

The envelope comprises a small amount of lipid of cellular origin and three hepatitis B surface proteins (HBs): large (LHB), medium, (MHB), and small (SHB), which form disulfide-linked homo- and heterodimers. The serum of infected individuals contains in addition, two types of sub viral particles: small spherical particles with a diameter of approximately 20 nm and filamentous particles also with a diameter of about 20 nm but of variable length. These non-infectious sub viral particles lacking genomic DNA greatly outnumber the infectious viral particles and have a composition similar to that of the viral envelope (Kann, 2002).

The nucleocapsid is formed by multiple copies of core protein. Of the total 183–185 amino acids (depending upon genotype), the *N*-terminal 149–151 amino acids are responsible for self-assembly of the nucleocapsid. Although the steps in its assembly remain to be clarified, the first step is the formation of homodimers linked by disulfide bridges. The nucleocapsid contains pores that allow the diffusion of nucleotides during the synthesis of the DNA genome. The *C*-terminal amino acids of the core protein play a role in the packaging of the pregenome–polymerase complex within the nucleocapsid (Bruss, 2007).

2.2 Hepatitis B Virus Genomic Organization

HBV has a partially double-stranded but not covalently closed circular DNA genome composed of between 3182–3248 nucleotides, depending on the genotype. The genome consists of a complete minus-DNA strand with a short-terminal redundancy, and a shorter plus-DNA strand that leaves a single-stranded gap of variable length in mature nucleocapsids and released viruses (Kann, 2002; Jilbert *et al.,* 2002). Base-pairing of plus- and minus-strands in the cohesive overlap region of the genome maintains the

circular configuration. The 5′end of the negative -strand is covalently linked to the *N*-terminal portion of the viral polymerase. At its 5′end, the positive -strand is linked to a capped RNA oligonucleotide that is derived from the 5′end of the RNA pregenome and serves as the primer for plus-strand-DNA synthesis (Kann, 2002; Jilbert *et al.*, 2002).

The genome consists of four partially overlapping open reading frames (ORFs) (Kann, 2002) that express surface, precore/core, polymerase, and X proteins. Each ORF overlaps at least one other ORF, with the polymerase ORF overlapping all of the others, and every nucleotide is part of at least one ORF. Translation of preS1, preS2, and S ORFs leads to the expression of the surface proteins, LHB, MHB, and SHB (Kann, 2002; Jilbert *et al.*, 2002).

Figure 2.2: The Genome Organization of the HBV

Source: Locarnini, 2004

The genome of HBV is made up 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). The negative-sense (non-coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (−) sense strand and a short sequence of RNA from the (+) sense strand.

Non-coding bases are removed from the ends of the (−) sense strand and the ends are rejoined. There are four known genes encoded by the genome, called *C, X, P*, and *S*. The core protein is coded for by gene *C (HBcAg),* and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. In some rare strains of the virus known as Hepatitis B virus pre core mutants, no HBeAg is present (Buti *et al.*, 2005). 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 frame "start" (ATG) codons that divide the gene into three sections, pre-*S*1, pre-*S*2, and *S*. Because of the multiple start codons, polypeptides of three different sizes called large (the order from surface to the inside: pre-S1, pre-*S2,* and *S*), middle (pre-*S2, S*), and small (S) are produced (Glebe and Stephan, 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).

HBx enhances HBV replication, likely through both direct and indirect mechanisms, and usually by cooperating with the cellular signal-transduction machinery (Chen *et al*., 2005). HBx resides in the cytosol and nucleus of HBV-infected cells where it can modulate numerous cellular signal-transduction pathways and interact with various cellular proteins (Seeger and Mason, 2000).

The most compelling evidence that HBx is critical for the virus life cycle comes from two animal models that more closely recapitulate the HBV life cycle. In the first animal model, intrahepatic inoculation of woodchuck hepatitis virus (WHV) genomes capable of expressing woodchuck X protein (WHx) led to replication in woodchuck hosts, whereas WHx-deficient genomes did not replicate and the animals remained susceptible to subsequent viral challenge (Lee *et al*., 2003; Zoulim *et al*.,2006). In the second animal model, immunodeficient mice transplanted with human hepatocytes can be infected with HBV (Lee *et al*., 2003; Zoulim *et al*.,2006). Virus generated by transfection of HepG2 cells with a plasmid HBV DNA encoding HBx or a plasmid HBV DNA containing an HBx mutation that prevented HBx expression were used to infect human-liver chimeric mice (Seeger and Mason, 2000).

The requirement for HBx for HBV replication is most convincing in model systems that recapitulate the virus life cycle in vivo. and the impact of HBx on HBV replication is modest when virus replication is driven from a plasmid DNA template transfected into established cell lines, which could reflect the constitutive activation of various cellular-signaling pathways in these cell lines that HBx must activate in normal hepatocytes in vivo (Seeger and Mason, 2000).

2.2.1 Functions of the gene products

2.2.1.1 Surface proteins

The HBV surface protein: small (SHB), medium (MHB) and large (LHB), together with cellular lipid material, form the viral envelope (Kann, 2007). SHB antigen which represents 85% of hepatitis B surface antigen (HBsAg), is highly immunogenic and provokes the host's immune response to HBV. Excess surface protein circulating in sub viral particles is thought to dilute the host's immunological response to the virus (Kann, 2007).

LHB, in contrast to MHB, is essential for infection and viral morphogenesis. It represents 10–30% of the HBsAg of virions and filaments. LHB plays a role in viral entry into hepatocytes, although SHB may also be needed in this process (Kann, 2007).

2.2.1.2 Core protein and 'e' antigen

Core protein (C) is the major structural component of the nucleocapsid. The pre C/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the *N*-terminal end of the precore protein by a signal peptidase. When transported into the Golgi compartment, additional amino acids are removed from the *C*-terminal end by intra-Golgi proteases to form HBe antigen. This antigen is secreted into the serum. The biological function of HBe remains unsolved (Kann, 2007).

2.2.1.3 Polymerase protein

Polymerase (P) has four domains: a terminal domain, which serves as a protein primer for reverse transcription of pregenomic viral RNA; a spacer region without apparent function; the polymerase domain, which has reverse transcription activity; and the

RNase H domain, which is responsible for the degradation of the RNA template during reverse transcription (Kann, 2007).

2.2.1.4 X- protein

The hepatitis B virus (HBV) is a major cause of human hepatocellular carcinoma (HCC) which has a very high mortality rate due to high incidence of metastasis. The hepatitis B Virus X protein promotes hepatocellular carcinoma metastasis by upregulation of matrix metalloproteinases (Chi *et al*., 2014). The hepatitis B virus (HBV) X protein (HBx) is a transcriptional transactivation that is essential for viral replication and has been implicated in the development of HBV-related hepatocellular carcinoma (Chi *et al*., 2014). Although not binding itself to DNA, it regulates transcription from HBV promoters, and from the promoters of cellular genes, including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, and cellular adhesion. HBx also forms complexes with several signal transduction proteins and regulators of cell growth and survival and plays a central role in HBV regulation and pathogenesis (Feitelson *et al*., 2005; Benhenda *et al.,* 2009).

Wang, *et al*. (2011) reported that natural HBx mutants might be selected in tumor tissues and play a role in hepatocarcinogenesis by modifying the biological functions of HBx.

The HBV X-protein, a multi-functional viral regulator, has been suspected to play a positive role in hepatocarcinogenesis. Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-gamma and hepatitis B Virus X protein in hepatocellular carcinoma cells. MicroRNA-122 (miR-122), a pivotal liver-specific

miRNA, has been implicated in several liver diseases including hepatocellular carcinoma (HCC) and hepatitis C and B viral infection.

HBx has been shown to have a variety of biological functions, including transcriptional activation of a variety of viral and cellular promoters, its interaction with p53, interference with host DNA repair, repression of physiological proteolysis, modulation of cell proliferation and apoptosis, and induction of malignant cell migration. (Wang *et al.,* 2011; Howard, 2017) These functions may contribute to the initiation and development of HCC associated with a hepatitis B virus (HBV) infection.

The invasion and metastasis of cancer cells is a multi-step process (Chi *et al*., 2014) HCC frequently invades blood vessels early and metastasize intra-hepatically and later extra-hepatically, during which, the destruction of the extracellular matrix (ECM), including the basement membrane, is an essential initial step because the ECM surrounding tumor tissues and the basement membrane is normally a barrier against cancer invasion (Chi *et al*., 2014). Previous studies have shown that MMP-2 (a gelatinase A/72-kD type IV collagenase) might play an important role in HCC invasion and metastasis (Chi *et al*., 2014).

2.3 Viral life cycle

During both acute and persistent infection, high levels of infectious HBV particles (virions) circulate in the bloodstream, together with an excess of empty particles (Seeger and Mason, 2000). Hepatocytes, the major targets of the virus, are separated from the bloodstream by endothelial and Kupffer cells that line the sinusoids of the liver. Liver sinusoidal endothelial cells have long cytoplasmic components that contain fenestrations with a diameter of 50–100 nm. Virions are thought to pass through these

fenestrations from the sinusoids of the liver to the space of Disse, which is immediately adjacent to the surface of the hepatocytes. Infectious virions bind by means of the PreS1 domain of LHBs and perhaps by the envelope lipid to specific, as yet unidentified, receptors on the hepatocyte surface (Seeger and Mason, 2000; Jilbert *et al*., 2002; Beck and Nassal, 2007; Kann *et al*., 2007).

HBV then proceeds following a characteristic replication strategy shared by all the members of the hepadnaviridae family (Seeger and Mason, 2000; Jilbert *et al*., 2002; Beck and Nassal, 2007; Kann *et al*., 2007). The nucleocapsid is released into the cytoplasm and translocated by microtubules to the microtubule-organizing centre (MTOC) near the nucleus. How the nucleocapsid gets from the MTOC to the nucleus is not known at the time of writing. Access to the nucleus is gained through nuclear pores (Kann *et al*., 2007). Nuclear import (Importin-β), which is a type of karyopherin, transports the HBV genome into the nucleus by binding to the C terminus of the nuclear pores (Kann *et al*., 2007) and may be mediated by polymerase or heat shock proteins. The exact stage and mechanism by which the viral genome is released from the nucleocapsid is not currently known. In the nucleus, rc-DNA is converted into cccDNA, the key template in HBV replication. The steps in achieving this conversion are uncertain, but they include completion of the positive DNA strand by polymerase; removal of the covalently linked polymerase, the 5′capped oligonucleotide primer, the terminal redundancy, and ligation of the 5′ and 3′ends of the positive and negative DNA strands.

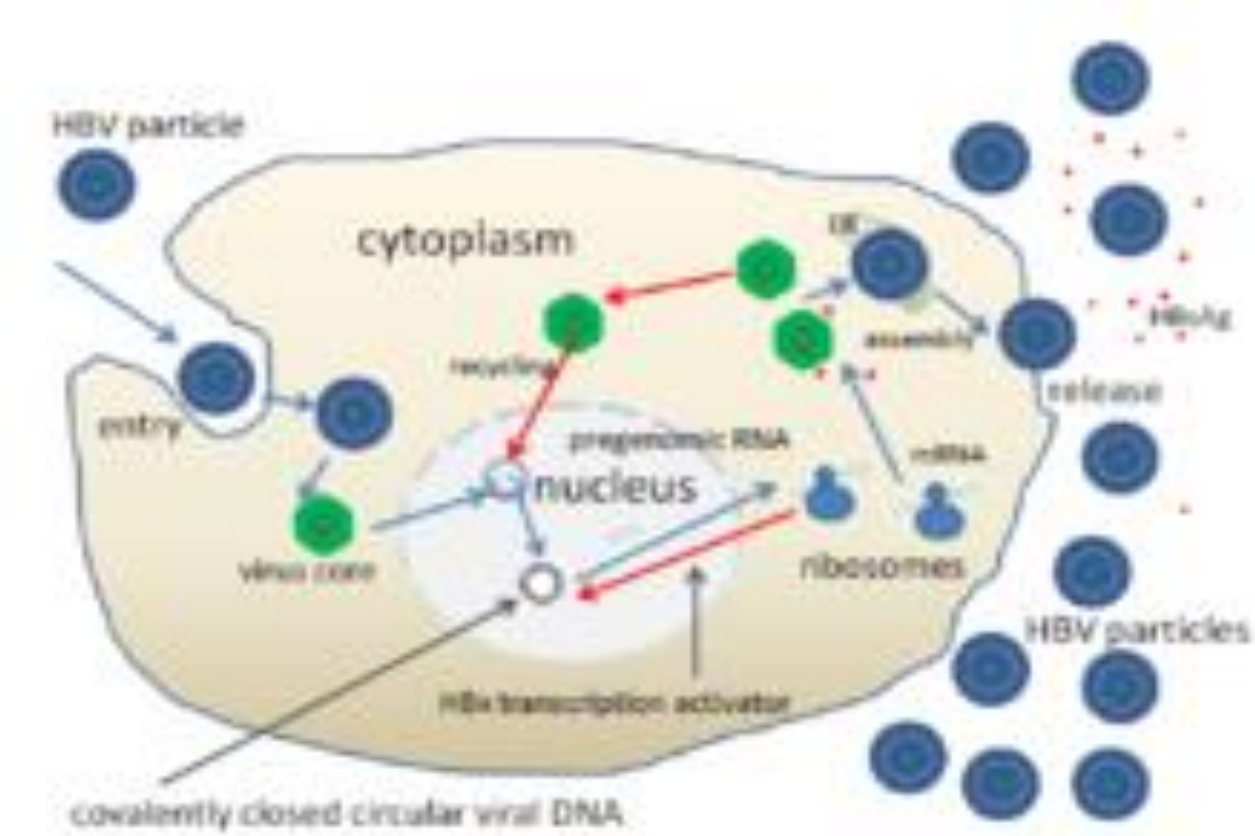

Figure 2.3: Hepatitis B Virus Replication Process

Source: Yan *et al.,* 2012

The life cycle of hepatitis B virus is complex. Hepatitis B is one of a few known pararetroviruses non retroviruses that still use reverse transcription in their replication process. The virus gains entry into the cell by binding to NTCP on the surface and become endocytosed (Yan *et al.*, 2012). 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 by a viral polymerase and transformed into covalently closed circular DNA (cccDNA). This cccDNA serves as a template for transcription of four viral mRNAs by host RNA polymerase. The largest mRNA, which is longer than the viral

genome, is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions that are released from the cell or returned to the nucleus and re-cycled to produce even more copies. (Beck and Nassal, 2007) The long mRNA is then transported back to the cytoplasm where the virion P protein (DNA polymerase) synthesizes DNA via its reverse transcriptase activity.

2.4 Target Cells

HBV is primarily hepatotropic virus, and hepatocytes are the only confirmed site of replication for all members of this virus family. Although the virus has been detected in other cells such as bile duct epithelial cells, peripheral blood mononuclear cells and cells in the pancreas and kidneys, the evidence for viral replication in these cells is controversial (Seeger and Mason, 2000). The reason for the high specificity is that HBV recognizes distinctive characteristic of the hepatocyte cell surface, including highly sulfated heparan sulfate proteoglycans (HSPGs) (Pourkarim *et al*., 2010; Cao, 2009), which help to confer tissue specificity and are formed from several heparan sulfate glycosaminoglycan chains which are abundantly expressed on the surface of cells and in the extracellular matrix (Pourkarim *et al*., 2010; Nassal, 2007).

2.5 Prevalence and Geographic distribution of Hepatitis B Virus Genotypes

The virus is divided into eight major genotypes (A–H). 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. (Beck & Nassal, 2007). Genotypes differ by at least 8% of their sequence and were first reported in 1988

when six were initially described (A–F) (Li *et al*., 2010; Yan *et al.*, 2012). Genotypes A, D and F have been, recently, divided into four subtypes (adw, adr, ayw, and ayr) (Kramvis *et al*., 2005). They appear to be associated with particular geographic distribution, ethnicity, and possibly clinical outcomes with a difference ranging from 4 to 8% in their nucleotide sequences (Günther, 2006; Mc-Mahon, 2009).

The mechanism that allows translation from the downstream polymerase protein start codon may include a direct internal ribosomal entry-site-like binding of the ribosomal subunits at, or near to, the polymerase start codon, "leaky' scanning of the ribosomes that allow passage to the downstream start codon, or the presence of a 'minicistron' upstream of the polymerase ORF that is translated (Jilbert *et al*., 2002).

There is a wide variation of HBV infection in the world (Custer *et al*., 2004; CDC, 2012). Approximately 45% of the world population lives in areas where chronic HBV infection is highly endemic > 8% of the population are Hepatitis B Surface Antigen (HBsAg) positive; 43% live in areas where endemicity is intermediate 2–7% Hepatitis B Surface Antigen (HBsAg) positive; and 12% live in areas where endemicity is low < 2% Hepatitis B Surface Antigen (HBsAg) positive (CDC, 2012). The prevalence of chronic HBV infection is lowest in North America, Northern and Western Europe, Australia and New Zealand; intermediate in Japan, the Middle East, Eastern and Southern Europe and parts of South America; and highest in sub-Saharan Africa, the Amazon Basin, the People's Republic of China, the Republic of Korea, Taiwan, and several other countries in South-east Asia (Chen, *et al*., 2000; Custer *et al*., 2004).

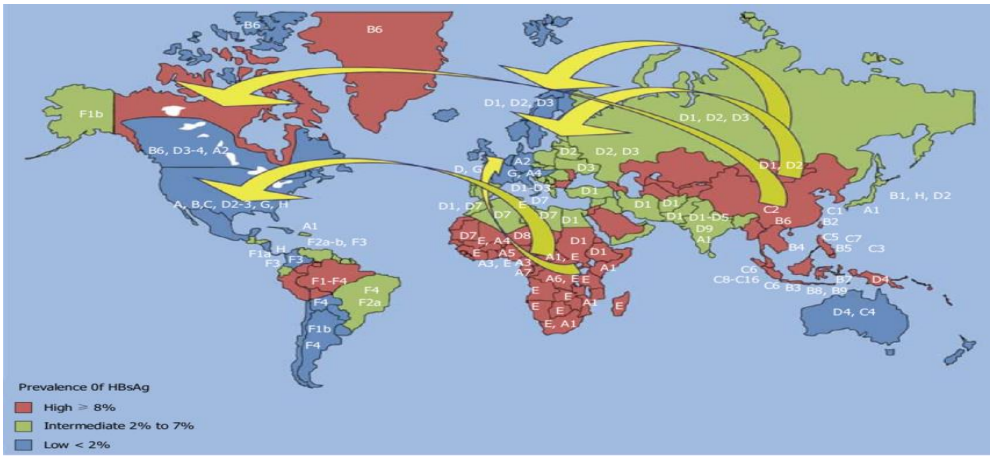

Figure 2. 4: Prevalence of Hepatitis B Virus Infection

Source: GBD, 2016.

An estimated 350 million individuals were infected worldwide. National and regional prevalence range from over 10% in Asia to under 0.5% in the United States and Northern Europe.

The primary method of transmission reflects the prevalence of chronic HBV infection in a given area. In low prevalence areas such as the continental United States and Western Europe, injection drug abuse and unprotected sex are the primary methods, although other factors may also be important (WHO, 2011). In moderate prevalence areas, which include Eastern Europe, Russia, and Japan, where 2–7% of the population is chronically infected, the disease is predominantly spread among children. In high-prevalence areas such as China and South East Asia, transmission during childbirth is most common, although in other areas of high endemicity such as Africa, transmission during childhood is a significant factor (GBD, 2016). The prevalence of chronic HBV infection in areas of high endemicity is at least 8% with 10–15% prevalence in Africa/Far East (WHO, 2011). As of 2010, China has 120 million infected people, followed by India and Indonesia with 40 million and 12 million, respectively. According to World Health

Organization (WHO, 2011), an estimated 600,000 people die every year related to the infection.

The worldwide variation in the endemicity of HBV infection is influenced primarily by the predominant age and the modes of transmission by which it occurs. In areas of high endemicity, the lifetime risk of HBV infection is more than 60%, and most infections are acquired from perinatal and child-to-child transmission, acute hepatitis B is uncommon because most perinatal and early childhood infections are asymptomatic (WHO, 2011). Hepatitis B virus (HBV) infection in infants and children is associated with few or no symptoms but poses a high risk of becoming chronic (WHO, 2011). However, rates of liver cancer and cirrhosis in adults are very high. Chronic carriage is thought to result from vertical transmission in China, Taiwan (China), and the Republic of Korea (Chen *et al*., 2007). HBV infection in newborns is less common in Africa. A lower prevalence of HBsAg positivity has been observed in mothers from sub-Saharan Africa compared with mothers in Asia. Child-to-child horizontal transmission accounts for high hepatitis B infection in this region of Africa (Chen *et al*., 2007).

In areas where endemicity is intermediate, mixed patterns of transmission exist, including infant, early childhood, and adult transmission (Chen *et al*., 2007). In low endemicity areas, most HBV infections occur in adolescents and young adults with relatively well defined high-risk groups, including injection drug users, homosexual males, health care workers, and patients who require regular blood transfusion or hemodialysis. In countries where adult horizontal transmission patterns are the principal transmission routes, the incidence of HBV infection is highest in adults (Custer *et al*., 2004).

HBV is a prototype member of the hepadnavirus family. Currently, eight genotypes of HBV (A through H) have been identified on the basis of greater than 8% nucleotide divergence over the whole genome (Drexler *et al*., 2013; Iannacone *et al*., 2005). Genotype A is prevalent in Europe, Africa, and North America. Genotype B is prevalent in Taiwan, China, Thailand, South-east Asia, and genotype C is prevalent in China, Japan, the Republic of Korea, and South-east Asia. Genotype D is predominant in India, Mediterranean areas, and the Middle East region. Genotype E is limited to West Africa. Genotypes F and G are mostly found in Central and South America. Genotype H has been observed in Mexico and Central America (Custer *et al*., 2004; Stoyan *et al*., 2009).

Compared with patients infected with the HBV genotype B, those infected with genotype C have a significantly lower rate of spontaneous HBeAg seroconversion (Furusyo *et al*., 2002; Kao *et al*., 2004), a higher histological activity index of necroinflammation or fibrosis score (Lindh *et al*., 1999; Chan *et al*., 2003 Kobayashi *et al*., 2002, Lee *et al*., 2003) and a higher risk of developing acute exacerbations (Chu *et al*., 2005; Kao *et al*., 2004; Schilsky, 2013), reactivation of HBV (Chu *et al*., 2005; Liaw, 2005), end-stage liver disease (Kao *et al*., 2004; Chan *et al*., 2003; Chu and Liaw, 2005) and Hepatocellular carcinoma (Yu *et al*., 2005; Yang *et al*., 2008).

2.6 Pathogenesis of Hepatitis B Virus Infection

Hepatitis B virus primarily interferes with the functions of the liver by replicating in hepatocytes (Yan *et al*., 2012). There is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D (Bruss, 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 extra hepatic sites,

suggesting that cellular receptors for HBV may also exist on extra hepatic cells (Bruss, 2007). 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. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes (Kramvis *et al*., 2005). 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 (Kramvis *et al*., 2005).

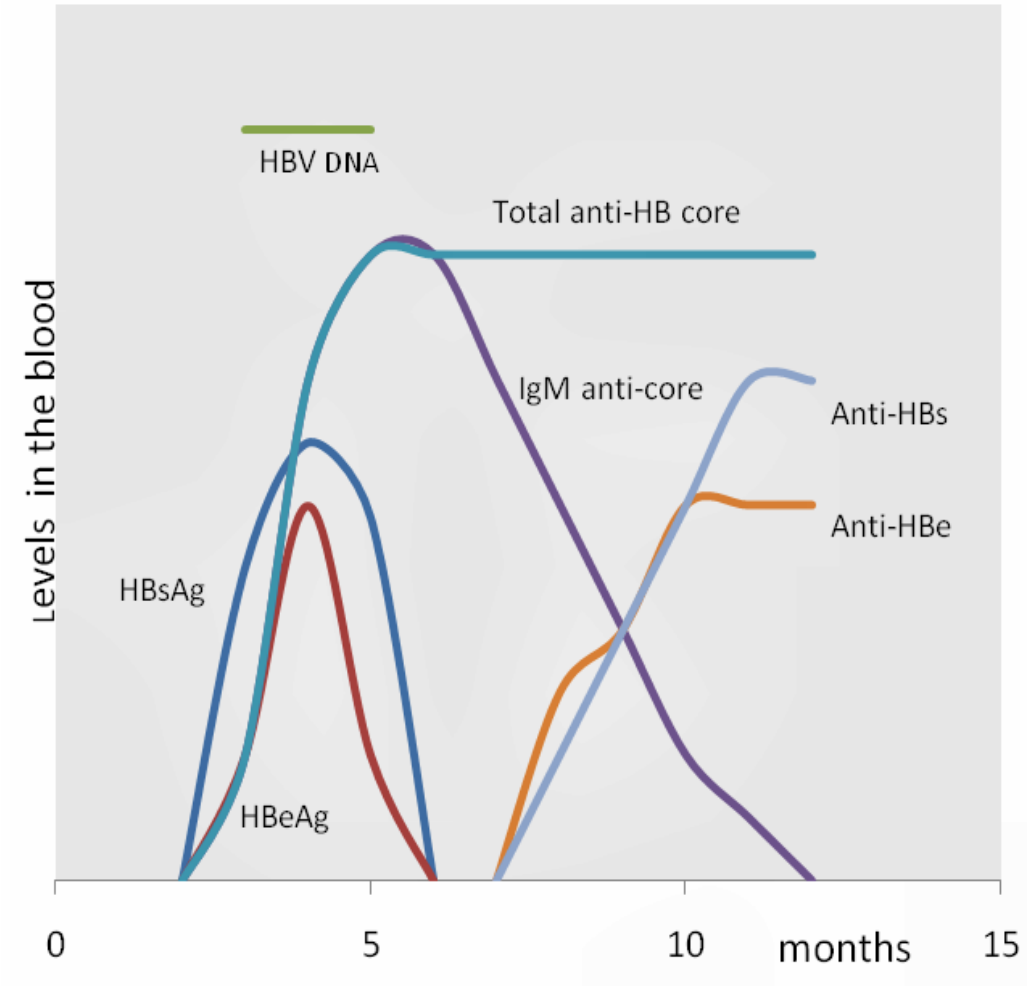

Figure 2.5: HBV Viral Antigens and Antibodies Detectable in Blood Stream Following Acute Infection

Source: Schillie *et al.*, 2017.

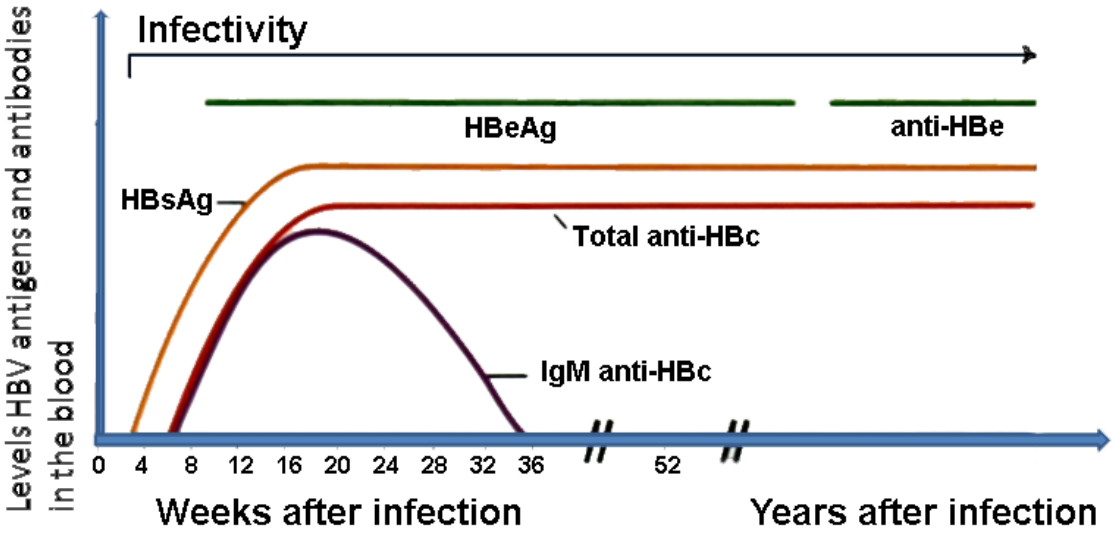

Figure 2.6: HBV Viral Antigens and Antibodies Detected in Blood Stream of a Chronically Infected Person.

Source: Schillie *et al.*, 2017

2.7 Diagnosis of Hepatitis B Virus

The tests for the 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 (Schillie *et al.*, 2017).

2.7.1 Hepatitis B Surface Antigen Test

The hepatitis B surface antigen test shows if a patient is contagious. A positive result means a patient have hepatitis B and can spread the virus. A negative result means a patient does not currently have hepatitis B. This test doesn't distinguish between chronic and acute infection. The hepatitis B surface antigen is most frequently used to screen for the presence of this infection (Araujo, 2015). 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 (Araujo, 2015).

2.7.2 Hepatitis B Core Antigen Test

The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of the core protein, alternatively known as hepatitis B core antigen. The hepatitis B core antigen test shows whether a patient is currently infected with HBV. Positive results usually mean a patient have acute or chronic hepatitis B. It may also mean a patient is recovering from acute hepatitis B. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies specific 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) (Araujo, 2015).

Shortly after the appearance of the HBsAg, another antigen called hepatitis B e antigen (HBeAg) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true (Araujo, 2015). 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.

2.7.3 Hepatitis B Surface Antibody Test

Hepatitis B surface antibody test is used to check for immunity to HBV. If the host is able to clear the infection, eventually the HBsAg will become undetectable and this will

be followed by IgG antibodies to the hepatitis B surface antigen and core antigen (*anti-HBs* and *anti HBc IgG*) (Zukerman and Baron, 2009). 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 may have cleared an infection or has been vaccinated previously.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Li *et al*., 2010). 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, if they are in the immune clearance phase of chronic infection. Carriers who have sero-converted 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 (Li *et al*., 2010). However, it is possible for individuals to enter an "immune escape" with HBe-Ag negative hepatitis.

2.7.4 Polymerase Chain Reaction (PCR) test

PCR tests have been developed to detect and measure the amount of HBV- DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim, 2006).

Quantification Hepatitis B virus (HBV) PCR test which reflects the viral load, plays a critical role in the management of chronic HBV infections (WHO, 2015). HBV DNA levels change during different phases of infection in chronically HBV infected individuals (WHO, 2015).

HBV DNA measurements (PCR tests) play a critical role in determining the phase of infection, deciding the treatment, and detecting responses to the antiviral therapy. (EASL, 2012)

According to the guidelines for the prevention, care, and treatment with persons with chronic hepatitis B virus from the World Health Organization (WHO, 2015). HBV DNA quantification is recommended in the treatment of chronic HBV infections. Real-time PCR, with its increased accuracy, wider linear range, and reproducibility, is widely used for the quantitative detection of HBV DNA (Mackay *et al.*, 2016; Allice *et al.*, 2007)

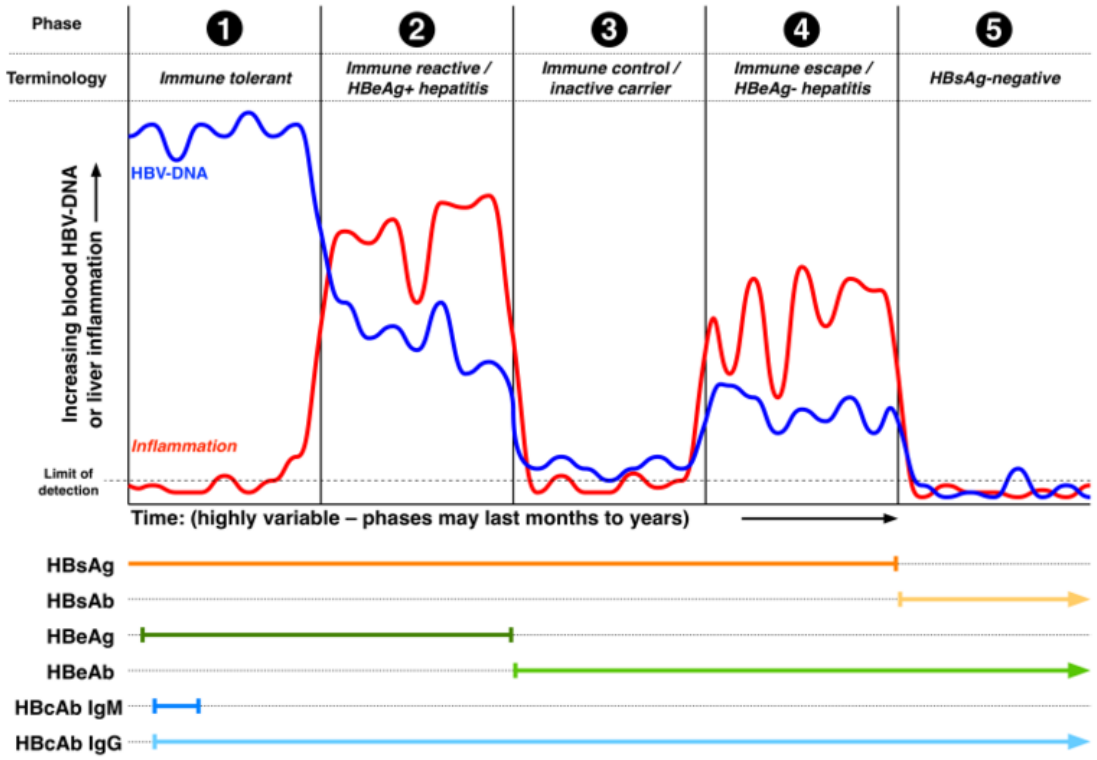

Figure 2.7: The five phases of chronic hepatitis B virus infection as defined by the European association for the study of the liver.

Source: Schillie *et al.*, 2017

2.7.5 Liver Function Tests

Liver function tests, also referred to as a hepatic panel, are groups of blood tests that provide information about the state of a patient's liver (Araujo, 2015). Commonly used tests to check liver abnormalities are: alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).

Alanine transaminase (ALT) is an enzyme that is involved in the metabolism of proteins. A damaged or malfunctioning liver may result in the release of ALT into the blood, which will cause ALT levels to rise. The normal range for ALT in the blood is 7 - 55 units per liter (U/L). Any value above this is an indication of liver damage. ALT values above 25 IU/L (international units per liter) for females and 33 IU/L for males indicates the need for further testing and evaluation, according to the American College of Gastroenterology. The activity of ALT in the serum is measured by coupling the reaction catalyzed by the enzyme to a reaction in which NADH is used up resulting in change in the photometric intensity when read in the UV range at 340 nm in a spectrophotometer (Araujo, 2015).

Aspartate aminotransferase (AST) is an enzyme that is also involved in the metabolism of proteins in the heart, liver, and muscles. AST is not specific for liver damage, but it is measured together with ALT to check for possible liver problems. When the integrity of the liver is compromised, AST can be released into the bloodstream. The normal range for AST in the blood is 40 IU/L for adults, but may be higher in infants and young children (Araujo, 2015).

The activity of AST in the serum is measured by coupling the reaction catalyzed by the enzyme to a reaction in which NADH is used up resulting in change in the photometric intensity when read in the UV range at 340 nm in a spectrophotometer.

Alkaline phosphatase (ALP) is an enzyme found in the bones, bile ducts, and liver. An ALP test is usually done in combination with several other tests to establish abnormalities in any of any of the above listed tissues. Abnormal levels of ALP may indicate liver inflammation, blockage of the bile ducts, or a bone disease. The normal range for ALP is ordinarily up to 120 U/L in adults, but children and adolescents may have elevated levels of ALP because their bones are growing. Pregnancy can also raise ALP levels (Araujo, 2015).

ALP is a hydrolase enzyme that removes phosphates from proteins, nucleotides etc. The method for determining the level of alkaline phosphatase is colorimetric; using p-nitrophenyl phosphate as substrate for the enzyme and measuring rate of formation of the colored product, p- nitrophenol. This measurement of the colour intensity is done at a wavelength of 405 nm (Araujo, 2015).

2.8 HBV Epidemiology

HBV is one of the most common infectious viruses worldwide (Chen *at al*., 2007). It is estimated that more than two billion people are infected and approximately 360 million of these are chronically infected (Lee *et al*., 2003; Chen *at al*., 2007; Dienstag, 2008). Approximately, one million people die each year from HBV-related chronic liver diseases, including liver cirrhosis and hepatocellular carcinoma (Mahoney, 1999). Chronic HBV infection is responsible for 50–90% of HCC in high-risk areas and HCC is one of the most common cancers in the world (Chen *at al*., 2007).

2.9 Signs and Symptoms of Hepatitis B Virus Infection

The symptoms of hepatitis B range from mild to severe and usually appear about one to four months after being infected, although symptoms may occur as early as two weeks' post-infection. Acute infection with hepatitis B virus is associated with an illness that begins with general ill-health, loss of appetite, nausea, vomiting, mild fever, dark urine, joint pain, weakness and fatigue (WHO, 2014). It has been noted that body aches have been an indication as a possible symptom of all hepatitis virus types, and the symptoms later progresses to yellowish skin and eyes (jaundice). The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have a more severe form of liver disease known as fulminant hepatic failure and may die as a result. The infection may be entirely asymptomatic and may go unrecognized (Terrault *et al.*, 2005).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (HCC; liver cancer). Across Europe, hepatitis B and C cause approximately 50% of hepatocellular carcinomas (El- Serag, 2011). Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of membranous glomerulonephritis (MGN) (Gan *et al.*, 2005). Symptoms outside of the liver are present in 1–10% of HBV-infected people and include serum-sickness–like syndrome, acute necrotizing vasculitis (polyarteritis nodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood (Gianotti–Crosti syndrome)

(Dienstag, 1981). The serum sickness like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice (Alberti and Caporaso, 2011).

The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice but can persist throughout the duration of acute hepatitis B (Liang, 2009). About 30–50% of people with acute necrotizing vasculitis (polyarteritis nodosa) are HBV carriers (CDC, 2015). HBV-associated nephropathy has been described in adults but is more common in children (CDC, 2015). Membranous glomerulonephritis is the most common form (Liang, 2009). Other immune-mediated hematological disorders, such as essential mixed cryoglobulinamia and aplastic anemia have been described as part of the extrahepatic manifestations of HBV infection, but their association is not as well-defined; therefore, they probably should not be considered etiologically linked to HBV (Liang, 2009).

2.10 Transmission of HBV Infection

HBV is highly contagious and is transmitted by percutaneous and per mucosal exposure to infected blood and other body fluids such as semen and vaginal fluid (WHO, 2011). The highest concentrations of the virus occur in blood and wound secretions (WHO, 2011). Moderate concentrations of HBV are found in semen and vaginal fluid, and lower concentrations occur in saliva. HBV is not spread by air, food, or water. Common modes of transmission include mother-to-infant, child-to-child, unsafe injection practices and blood transfusions, and close contact. HBV may be detected in serum 30–60 days following infection and may persist for long period of time (WHO, 2011).

Perinatal transmission from HBsAg-positive mothers to their newborn infants (vertical) or transmission from one child to another (horizontal) is a major source of HBV infections in many countries where chronic HBV infection is highly endemic (WHO,

2011). Perinatal transmission usually happens at the time of birth; in-utero transmission is relatively rare, accounting for less than 2% of perinatal infections in most studies. There is no evidence that HBV can be spread by breastfeeding (Beasley *et al*., 1977). The risk of perinatal transmission depends on the HBeAg serostatus of the mother. The risk of HBV infection approximately ranges from 70–90% for HBeAg-positive mothers to 5–20% for HBeAg-negative mothers (Okada *et al*., 1976 and Beasley *et al*., 1977). The spread of HBV from child to child usually occur in household but may also occur in child daycare centers and schools (WHO, 2011). The most probable pathways of child-to-child spread involve contact of skin sores, small breaks in the skin, or mucous membranes with blood or skin sore secretions (Margolis *et al*., 1997). HBV may also spread because of contact with saliva through bites or other breaks in the skin, and as a consequence of the premastication of food (Mac-Quarrie *et al*., 1974; Scott *et al*., 1980; Hyun *et al*., 2017; Beasley and Huang, 1983; Williams *et al*., 1997).

The virus may spread from inanimate objects such as shared towels or toothbrushes, because it can survive for at least 7 days outside the body (WHO, 2011) and can be found in high titres on objects, even in the absence of visible blood (Petersen *et al.,* 1976; Bond *et al.,* 1981; Martinson *et al*., 1998). Among Gambian children aged 6 months to 5 years, a significant association was observed between HBV infection and the presence of bedbugs in each child's bed (Vall-Mayans *et al*., 1990). But controlling bedbugs by insecticide spraying of the child's dwelling did not have any effect on HBV infection (Vall-Mayans *et al*., 1990).

Sharing of objects, the re-use of contaminated equipment for medical, cosmetic or dental procedures, failure to use appropriate disinfection and sterilization practices for equipment and environmental surfaces, and improper use of multidose medication vials,

can result in the transmission of HBV in many developing countries (Kann *et al*., 2007; Simonsen *et al*., 1999). Blood transfusion is also a common source of HBV transmission in countries where the blood supply is not screened for HBsAg. In addition, the injection of illicit drugs using shared syringes and needles is a common mode of HBV transmission in many developed countries.

HBV is efficiently transmitted by sexual contact, which accounts for a high proportion of new infections among adolescents and adults in countries with low and intermediate endemicity of chronic HBV infection (Alter and Margolis, 1990). Risk factors for sexual transmission include multiple sexual partners, prostitution, and lack of protection in sexual activity is a common mode of HBV transmission in many developing countries (CDC, 2017).

2.11 Prognosis of Hepatitis B Virus Infection

Hepatitis B virus infection may be either acute (self-limiting) or chronic (long-lasting). Persons with self-limiting infection clear the infection spontaneously within weeks to months (Kerkar, 2005). The hepatitis B virus usually only stays in the system for around one to three months and is therefore an acute infection. The infection usually resolves without treatment and most adults recover completely. However, in around 1 in 20 affected adults, the infection is chronic and stays in the body for six months or more.

This chronic form of hepatitis B is very common in young infants and children. About 90% of infants who are infected as newborns and 20% of young children affected go on to develop chronic infection. Even if these children do not have symptoms, they can still pass the infection onto other people (Kerkar, 2005). About 20% of people who develop long-term infection will develop liver disease and suffer from scarring or cirrhosis of the

liver. Around 10% of those who develop cirrhosis will go on to develop liver cancer (Oliveri *et al.,* 1991).

2.11.1 Cirrhosis of the Liver

Cirrhosis of the liver can take up to 20 years to develop and may not cause any symptoms initially. Eventually, the liver damage can lead to symptoms such as weakness, loss of appetite, nausea, vomiting, itchy skin, jaundice, pain in the right upper abdomen, and fluid buildup in the abdomen (ascites). A number of different tests are available to determine the degree of cirrhosis present. Transient elastography (Fibro Scan) is the test of choice, but it is expensive (WHO, 2015). Aspartate aminotransferase to platelet ratio index may be used when cost is an issue (WHO, 2015).

2.11.2 Liver Cancer

Around 10% of individuals with liver cirrhosis go on to develop liver cancer. The symptoms of liver cancer include weight loss, loss of appetite, nausea, vomiting, and jaundice (Taylor, 2006).

2.11.3 Fulminant Hepatitis B Virus

This is a rare complication of acute hepatitis B where the immune system attacks the liver and causes severe damage. The condition affects around 1 in 100 adults who have chronic hepatitis B, but children are affected much more rarely. Some of the symptoms of fulminant hepatitis B include confusion, jaundice and ascites (WHO, 2014).

2.11.4 Reactivation

Hepatitis B virus DNA persists in the body after infection, and in some people the disease recurs (Vierling, 2007) Although rare, reactivation is seen most often following alcohol or drug use (Villa *et al*., 2011) or in people with impaired immunity (Katz *et al*.,

2008). HBV goes through cycles of replication and non-replication. Approximately 50% of overt carriers' experience acute reactivation. Males with baseline ALT of 200 UL/L are three times more likely to develop a reactivation than people with lower levels. Although reactivation can occur spontaneously (Roche and Samuel, 2011). People who undergo chemotherapy have a higher risk (Mastroianni *et al*., 2011). Immunosuppressive drugs may increase HBV replication while inhibiting cytotoxic T cell function in the liver (Buddeberg *et al*., 2008) the risk of reactivation varies depending on the serological profile; those with detectable HBsAg in their blood are at the greatest risk, but those with only antibodies to the core antigen are also at risk. The presence of antibodies to the surface antigen, which are considered to be a marker of immunity, does not preclude reactivation (Mastroianni *et al*., 2011). Treatment with prophylactic antiviral drugs can prevent the serious morbidity associated with HBV disease reactivation (Mastroianni *et al*., 2011).

2.12 Factors Modifying the Risk of Hepatitis B Virus Infection

2.12.1 Aflatoxins

Aflatoxins play a vital role in liver cancer etiology, especially among individuals who are carriers of HBsAg (IARC, 2002) and this is supported by a nested case–control study conducted by Qian *et al*., (1994). The odds ratio associated with urinary aflatoxin biomarkers was 3.4 (95%CI: 1.1–10), and for HBsAg positivity alone 7.3 (95%CI: 2.2–24.4). However, when these two risk factors were positive, the odds ratio was 59 (95% CI: 17–212), suggesting multiplicative effect modification.

Wu *et al*. (2008) reported results from two different nested case–control studies based on the same community-based Cancer Screen Program cohort in Taiwan, China. In one, they examined urinary 15-F_{2t}-isoprostane as an indicator of oxidative stress and showed

that it was correlated with urinary aflatoxin–albumin adduct levels. They found that higher levels of this marker increased the risk of HBV particularly in HBsAg-positive careers. In comparison to those with low urinary 15-F_{2t}-isoprostane and without HBV infection, those with chronic HBV infection and 15-F_{2t}-isoprostane above mean level had an odds ratio of 19.0 (95%CI: 6.7–54.2). In the second study, the association between exposure to polycyclic aromatic hydrocarbons (PAHs) and the risk of HBV was examined. The levels of PAH–albumin adducts appeared to modify the effect of aflatoxin and HBV infection.

Kirk *et al*. (2005) has explored the effects of high dietary exposure to aflatoxins on the risk of HBV in a case control study in Gambia, where HBV infection is highly endemic. In the first study, mutations of the *TP53* gene at codon 249 (a mutation associated with aflatoxin exposure) were measured in the plasma of HBV patients and of healthy subjects. The risk of HBV was found to be elevated in those HBsAg-positive (OR, 10.0; 95%CI: 5.2–19.6), in those 249 seropositive alone (OR, 13.2; 95%CI: 5.0–35.0), and when both markers were present (OR, 399; 95%CI: 48.6–3270).

In the second study, human DNA was analyzed for genetic polymorphisms in aflatoxin-metabolism and hence activating (GSTM1, GSTT1, HYL1*2) and DNA-repair (XRCC1) enzymes. Statistically significant associations were found for the null GSTM1 genotype (OR, 2.45; 95%CI: 1.21–4.95), and also for the combined metabolizing enzyme genotypes. The HBV risk was most prominent among the individuals with the highest groundnut consumption (OR, 4.67; 95%CI: 1.45–15.1). These data suggest susceptibility to HBV can be altered by aflatoxin, but do not clearly demonstrate an interaction of aflatoxin with HBV in carcinogenesis (Kirk *et al*., 2005).

2.12.2 Alcohol

The association between alcohol consumption and the risk of HBV has been reviewed recently by IARC in Volume 96 (IARC, 2010). The assessment was made difficult by the fact that signs and symptoms of cirrhosis often preceded the cancer, which may have led to a modification of alcohol intake.

In a cohort study of 11,893 men, Yang *et al*., (2008) found that while alcohol consumption was associated with HBV (RR, 1.5; 95%CI: 1.0–2.3), when individuals who were positive for HBsAg were stratified according to alcohol use status, the relative risk for HBV was 11.4% for men who drank alcohol, and 9.7% for men who did not drink alcohol.

In a study conducted by Evans *et al.,* (2002) a relative risk of 0.9 (95%CI: 0.8–1.0) was found for alcohol consumption of more than three drinks per week in men in a multivariable model with no interaction with HBsAg-positivity. In women, alcohol consumption had a relative risk of 0.6 (95%CI: 0.3–1.2) in the multivariable model, and again, no interaction with HBsAg positivity was shown.

In a cohort study of 1,283,112 men and women free of cancer in the Republic of Korea, Jee *et al*. (2004) reported that Heavy alcohol consumption in men was associated with a relative risk for HBV of 1.5 (95%CI: 1.2–2.0), but there was no interaction between alcohol drinking and HBsAg positivity.

Donato *et al*. (1997) found a positive correlation between self-reported history of heavy alcohol consumption and HBV infection. The relative risk for joint exposures (RR, 64.7; 95%CI: 20–210) was greater than the sum of the relative risks for HBsAg-

positivity (RR, 9.1; 95%CI: 3.7–22.5), and for alcohol intake alone (RR, 4.2; 95%CI: 2.4–7.4).

2.12.3 Smoking

Chang, (2007) found a small but statistically not significant increased risk of development of HBV associated with cigarette smoking (RR, 1.22; 95%CI: 0.55–2.71). The cohort study of Yang *et al*., (2008) found that when HBV-infected men (HBsAg-positive and HBeAg-positive) were stratified by their smoking status, the relative risk for HBV was higher among smokers (RR, 76.9; 95%CI: 39.4–150.3) than non-smokers (RR, 67.0; 95%CI: 26.1–171.7).

Evans *et al*. (2002) reported that smoking in men was not associated with an increased risk of HBV, whereas in women, smoking showed a dose–response trend with increasing cigarette consumption: 1 to 5 cigarettes per day, relative risk 1.5 (95%CI: 0.4–6.3); 6 to 10 per day, 2.0 (95%CI: 0.6–6.5) and 10 cigarettes per day, 4.2 (95%CI: 1.3–13.8).

Jee *et al*. (2004) also reported that cigarette smoking was associated with an increased relative risk for HBV mortality. However, the increase was in male smokers (RR, 1.4; 95%CI: 1.3–1.6), but not in women (RR, 1.1; 95%CI: 0.8–1.7).

2.12.4 Metabolic factors

Two of the cohort studies have reported on the effects of obesity; body mass index of ≥ 30 kg and diabetes on the risk of HBV. Chen *et al*. (2007) reported that obesity may be associated with a 4-fold risk of HBV in those who were anti-HBV-positive.

Diabetes was associated with an increased risk in those positive for HBsAg. In comparison to the referent group of individuals with no chronic HBV infections, no

diabetes, and low BMI (< 30 kg/m^2), for individuals with chronic HBV infections, diabetes, and obesity (BMI ≥ 30 kg/m^2), the relative risk was as high as 264.7 (95%CI: 35.2–1993).

Yu *et al*. (2008) reported that excess weight may increase the risk for HBV among HBsAg-positive men in Taiwan, China. In comparison to men with a normal weight, overweight men (BMI, 25–< 30 kg/m^2) had a relative risk for HBV of 1.48 (95%CI: 1.04–2.12), and obese men (BMI ≥ 30 kg/m^2), 1.96 (95% CI: 0.7–5.4).

2.13 Prevention of HBV Infection

2.13.1 Vaccination

The hepatitis B vaccine is recommended for Newborns, Children and adolescents not vaccinated at birth, those who work or live in a center for people who are developmentally disabled, People who live with someone who has hepatitis B, Health care workers, emergency workers and other people who come into contact with blood; anyone who has a sexually transmitted infection, including HIV, polygamous family setting, People who inject illegal drugs or share needles and syringes, People with chronic liver disease, People with end-stage kidney disease and travelers planning to go to an area of the world with high hepatitis B infection rate (Schillie *et al.,* 2017; Hughes, 2000).

Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 U/ml in the recipient's serum. The vaccine is more effective in children and 95% of those vaccinated have protective levels of antibody. Adult vaccinations are given as a series of three injections over six months (Schillie *et al.,* 2017; Seyed, 2006). Booster doses may be necessary if a blood test reveals that immunity against hepatitis B is not at the desired

level. The vaccine is 80-100% effective in preventing infection even after antibody levels fall below 10 mLU/ml (Schillie *et al.,* 2017). For newborns of HBsAg-positive mothers: hepatitis B vaccine alone, hepatitis B immunoglobulin alone, or the combination of vaccine plus hepatitis B immunoglobulin, all prevent hepatitis B occurrence (Schillie *et al.,* 2017). Furthermore, the combination of vaccine plus hepatitis B immunoglobulin is superior to vaccine alone (Schillie *et al.,* 2017). This combination prevents HBV transmission around the time of birth in 86% to 99% of cases (Araujo, 2015). Tenofovir given in the second or third trimester can reduce the risk of mother to child transmission by 77% when combined with hepatitis B immunoglobulin and the hepatitis B vaccine, especially for pregnant women with high hepatitis B virus DNA levels (Schillie *et al.*, 2017).

In 10 to 22-year follow-up studies, there were no cases of hepatitis B among those with a normal immune system who were vaccinated (WHO, 2014). Vaccination is particularly recommended for high risk groups including: health workers and people with chronic renal failure (WHO, 2014).

Both types of the hepatitis B vaccines, the plasma-derived vaccine (PDV) and recombinant vaccine (RV) are of similar effectiveness in preventing the infection in both healthcare workers and chronic renal failure groups (WHO, 2014). With one difference noticed among health worker group, that the RV intra-muscular route is significantly more effective compared with RV intra-dermal route of administration (Schillie *et al.*, 2017).

2.14. Treatment of HBV Infection

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously (Hollinger and Lau, 2006; CDC, 2017). Early antiviral

treatment may be required in less than 1% of people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immune-compromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of liver cirrhosis. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Lai and Yuen, 2007). Treatment lasts from six months to a year, depending on medication and genotype (Alberti and Caporaso, 2011). Treatment duration when medication is taken by mouth, however, is more variable and usually longer than one year (Tarrault *et al.*, 2016).

Although none of the available medications can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As of 2008, there were seven medications licensed for the treatment of hepatitis B infection in the United States. These include antiviral medications lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telmivudine (Tyzeka) and entecavir (Baraclude), and the two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys).

In 2015, the World Health Organization recommended tenofovir or entecavir as first-line agents (WHO, 2015). The use of interferon, which requires injections daily or thrice weekly, has been supplanted by long-acting PEGylated interferon, which is injected only once weekly (Dienstag, 2008). However, some individuals are much more likely to respond than others, and this might be due to the viral genotype. The treatment reduces viral replication in the liver, thereby reducing the viral load (Pramoolsinsup, 2002).

Response to treatment differs between the genotypes of Hepatitis B virus. 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 approximately 45% in genotypes A and B but only 25–30% genotypes C and D (Cao, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

Niger State is located in North Central Nigeria. The State lies on latitude 3.2^0 East and longitude 11.30^0 North, with Minna as the capital It is 150km away from the Federal Capital Territory, Abuja. Two of Nigeria's major hydroelectric power stations; the Kainji and the Shiroro dams, and an upcoming one in Zungeru are located in Niger State. The State has a population of 3,950,249 (National Population Commission, 2006). Niger State has three (3) senatorial zones with 25 local government areas. The three senatorial zones under study have three dominant ethnic groups; the Nupe, Gbagyi and Hausa respectively. The major farm products cultivated by these ethnic groups are rice, yam, millet and cassava, (Information Department of Ministry of Information, Communication and Integration, Minna, Niger State).

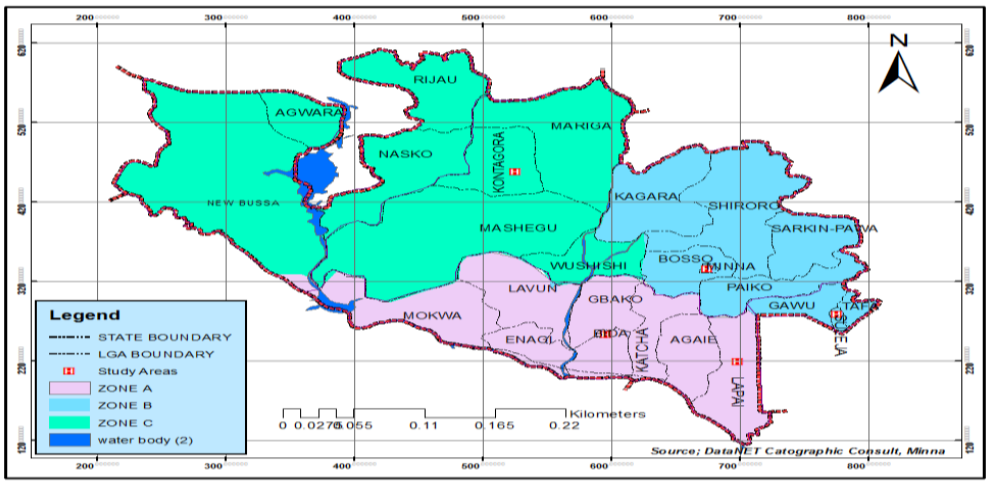

Figure 3.1: Map of Niger State indicating the study sites

Source: Anonymous (2019).

3.2 Sample Size Determination

The sample size for the research work was determined by the formula below.

$$n = \frac{Z^2 \mathrm{p}(1 - Z\mathrm{p})}{d^2}$$

(Daniel, 2020).

Where

n = required sample size

Z = confidence level at 95% (standard value of 1.96)

P = prevalence rate of the disease (10%)

d = marginal tolerable error at 5 % (standard value of 0.05)

Sample size, will be calculated using 10% prevalence obtained by Kuta *et al*., (2014) by substituting into the formula above.

$$S = \frac{1.962^2 \times 0.1\ (1 - 0.1)}{(0.05)^2}$$

$$S = \frac{00.3849444\ (0.9)}{0.0025}$$

S = 138.579984

S = 139.

Therefore, 139 samples were the minimum sample size for the research. 500 blood samples were collected in the ratio 1:1:1: 1:1 from the five selected hospitals.

3.3 Study Population and Scope

Blood samples were collected from 500 (male and female) patients, 18years and above, attending General Hospital Minna (GHM), General Hospital Kontagora (GHK), Umaru Sanda Ndayako Hospital Bida (USNHB), General Hospital Lapai (GHL) and General Hospital Suleja (GHS). 100 samples were collected from each hospital. The choice of hospitals was based on the fact that those hospitals serve as reference centers. A consent form was signed by each participant before the commencement of the collection of samples.

3.4 Ethical Approval

Approval to conduct this research was granted by the Ethics and Publications Committee of Niger State Hospitals Management Board (Appendix A). In accordance with the guidelines of the Ethics and Publications Committee. Patients were provided with consent forms to capture the following variables: age, gender, marital status, immunization status, blood transfusion and sharing of utensils.

3.5 Inclusion and Exclusion Criteria

Patients with established HBV and HIV infection were excluded from this study. Only apparently healthy individuals were included in the study.

3.6 Administration of Questionnaire on Patients

Questionnaires requesting for information on the following socioeconomic/risk factors; sharing of utensils, gender, blood transfusion, age, history of vaccination and occupation, were administered on the study population. (Appendix B).

3.7 Blood Sample Collection and Processing

Blood samples were collected aseptically from the patients attending some hospitals using 2ml syringe and transferred into EDTA vacutainer bottles. Sample preparation was done at the Molecular Biology Laboratory unit, SHAMRAS Diagnostics, Minna. The samples were centrifuged at 4000 rpm for 1 min. The sera obtained were transferred into non- EDTA containers and stored at -20°C until needed for further analysis.

3.8 Detection of Hepatitis B Surface Antigen

The HBs-Ag rapid test kit (Capital Bio: 300082) was used for the detection of Hepatitis B Surface Antigen in samples. The samples were thawed at room temperature for 1hr. The strip was immersed into the serum vertically for 5 -10 secs until absorbance was observed. The strip was removed and placed on non-absorbance flat surface and allowed to stand for 20 mins. The result was interpreted and observations were made in accordance with the manufacturer's instructions at the Molecular Biology Laboratory of SHAMRAS Diagnostics Hospital, Minna, Niger state. HBV positive samples were taken to the DNA Laboratory, Kaduna, Nigeria for further analysis.

3.9 Hepatitis B Virus DNA Extraction

DNAs in the samples were extracted using Automatic Nucleic Acid Extraction Instrument (Hunan Runmei Gene, NE 40) at the Molecular Laboratory of DNA laboratory, Kaduna, Nigeria. The protocol was designed for rapid automated extraction of viral nucleic acids from serum. 20µL of proteinase k, 20µL MagBind particles and 400µL Buffer MLB were added to a 1.5mL sterile micro centrifuge tube. 200µL of samples (serum) was transferred to the mix and vortexed for 15 secs and incubated at 25°C for 10 mins and during the process, the tubes were rotated up and down 3 times.

The tubes were placed on the magnetic rack for 10 mins until the MagBind particles have formed a tight pellet and the supernatants were then discarded.

500µL Buffer MW2 was added to each tube and vortexed for 10s, the tubes were placed on the magnetic rack for 3 mins until the MagBind particles have formed a tight pellet and the supernatants were discarded, the tubes were spinned and residual liquid was removed and air - dried for 3 minutes. 50µL of Nuclease - free water was added and the tubes were vortexed for 15secs. The samples were then incubated at room temperature for 5 mins. The tubes were spinned and placed on the magnetic rack for 2 mins until the MagBind Particles have formed a tight pellet; the supernatant containing the purified viral DNA was transferred to a 1.5ml micro centrifuge tube.

3.10 Hepatitis B Virus Amplification

The DNAs extracted from HBV positive samples were amplified at the facility of DNA laboratory, Kaduna, Nigeria. The HBV DNA amplification was carried out using the PCR Protocol as described by Chan *et al*., (2003). In the first round of amplification, the PCR reaction was performed using 10mM tris HCl (pH 9.0), 50mM KCl, 1.5 mM $MgCl_2$ 20µL of deoxynucleotide triphosphate (dNTPs), 0.01% gelatin, 3 units of Taq polymerase 0.2m M of primer and 4 µL of sample DNA. The DNA sample was placed along with all the components needed for the reaction into PCR tubes and placed into a terminal cycler. The amplification process consisted of initialization at 92-94°C for 2 minutes to activate the DNA polymerase and denatures other contaminants in the mixture, followed by denaturation at 94°C for 3 mins were the hydrogen bond between the double stranded DNA were broken to have single strands followed by annealing at 77°C for 30 secs and then elongation at 78°C for 1 min. Final elongation was done at

78°C for 15mins to ensure full elongation of the remaining DNA strands in the mixture. The cycle was repeated 35 times for 30 secs.

In the second round of amplification, 2 reactions were performed using 2 mixes of primers. (Table 3.1). For the mix A reaction, the followings were added to each lyophilized PCR tubes, 2µL of buffer containing 1.5mM $MgCl_2$ 0.4µL dNTPs, 0.5 µL each of the four primers 3 units of Taq polymerase and 2µL PCR products of the first round of amplification process, same was carried out for positive and negative controls, the sterile water was added to make a total reaction volume of 20µL.

Amplification mixture with mix B were exactly the same as those with mix A except for the primers of each mix, following the completion of the amplification, the PCR products from the second amplification, together with molecular weight standards were electrophoresed.

3.11 Agarose Gel Electrophoresis

After completion of the amplification, the PCR products were removed and ran on a gel. The tank was set on a flat surface and allowed to balance and the combs were inserted into the tanks.

1.5g of agarose gel was dissolved in 100ml of sodium borate buffer to make 1.5% gel and heated in an oven, 5µL of ethidium bromide was added and gently poured on the tank and was allowed to solidify for 30mins.

DNA was then loaded into each well; a ladder was added to the first well in order to know the size of the amplicon (PCR product). There are different types of marker depending on the size, there is 50bp marker, which can serve as marker for DNA fragments with size within 50-1000bp, 500bp (from 500-5000bp).

It was set on a power pack at 80 – 150v until the dye line was approximately 75 -80 % of the way down the gel and was allowed to run for 1 hr after which the power was switch off and the gel was carefully taken out and exposed to UV light. The picture of the gel was carefully taken with a gel documentation system (Gel Doc 2000, BIORAD, USA) (Appendix D).

The primers were obtained from DNA lab Kaduna as shown on table 3.1 below

Table 3.1: Primer Sequence Used

Primers	Sequence	Expected size (bp)
1ST Round Amplification		
hepBP1	5' – TCACCATATTCTTGGGAACAAGA–3'	
hepBS1 – 2	5' – CGAACCACTGAACAAATGGC–3'	
2ND Round Amplification		
MIX A		
hepB BAIR	5' – CTCGCGGAGATTGACGAGATG–3'	68
hepB BCIR	5' – GGTCCTAGGAATCCTGATGTTG–3'	281
hepB BBIR	5' – CAGGTTGGTGAGTGACTGGAGA–3'	122
Mix B		
hepB BE1	5' –CACCAGAAATCCAGATTGGGACCA–3'	169
hepB B2R	5' – GGAGGCGGATXTGCTGGCAA–3'	97
hepB BD1	5' – GCCAACAAGGTAGGAGCT–3'	119

3.12 HBV DNA Purification

The Standard protocol as described by Nakano *et al*., (2012) was followed for the purification of HBV DNA. PCR products from the first round of amplification were precipitated with 60µl ethanol (95%v/v), 3mM Sodium Acetate (pH 5.0) in the presence

of 5µL glycogen at 4°C. The mixture was immediately centrifuged at 14,000rpm for 15 mins. The supernatant was discarded, while the pellet was washed with 200µl of cold 70% (v/v) ethanol, and centrifuged at 14,000rpm for 2 mins at 4°C. Finally, the pellet was vacuum dried for 10 mins and dissolved in 40µl sample loading solution (provided in the kit). The suspended samples were transferred into the wells of the sample plates and overlaid with a drop of mineral oil.

3.13 Sequence Analysis

The Standard protocol as described by Edgar (2004) was followed. The purified DNA samples from 3.12 above were analyzed by sequencing using ANI automated sequencer (5µL) at the Inqaba Biotech, West Africa. The nucleotide sequences were viewed using software version 2.0 of chrome (Appendix E).

3.14 BLAST Analysis

The Standard protocol as described by Oehmen and Baxter (2013) was followed. The sequences obtained were submitted as query sequences to Basic Local Alignment Search Tool (BLAST) (online available program at http://www.ncbi.nlm.nih.gov/ BLAST. cgi) BLAST is an algorithm and program for comparing primary biological sequence information to obtain the definite length of the subject sequence and identify the similarity of HBV DNA sample sequences with the sequence previously reported in the database. Sequence of each genotype was retrieved for construction of phylogenetic tree (Appendix F).

3.15 Phylogenetic Tree Analysis

Phylogenetic tree from HBV sequence were generated by maximum composite likelihood algorithms, using MEGA6 software. Test of phylogeny that is, the percentage

replication of 1000 and branch values of >60%. The following were the accession numbers of different HBV reference sequences used in this study; genotype FN594754, LT823845, KY888216, HM180101, MF772435, KU702933, KY886216, EF662052, MG744344 (Tamura *et al*., 2011).

3.16 Data Analysis

HBV screening results of 500 patients from five General hospitals in five different locations in Niger state were considered. The data obtained in this study were subjected to statistical analysis using IBM SPSS 20.0 (SPSS Incorporation, 2007 Chicago, USA). Chi square test was carried out to check for significant differences in the incidence of hepatitis B virus in each of the five different locations; incidence among males and females in each of the locations; and prevalence of HBV infection in some hospitals in Niger state. Binary Logistic Regression analysis was also performed to ascertain if certain factors such as gender, marital status, history of vaccination sharing of utensils etc. contributed significantly to the likelihood that an individual tested positive to hepatitis B virus (Appendix H). Questionnaire responses of five hundred individuals from the five selected Hospitals in Niger state were also analyzed and the results obtained were used to correlate HBV infection with risk factors at 95% confidence interval and a p value ≤ 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seroprevalence of HBV infection in the study area

The prevalence of Hepatitis B virus infection amongst patients attending the five hospitals (GHM, GHS, GHK, GHL and USNHB) revealed that out of the 500 blood samples screened, 65 were positive representing 13.0% prevalence. Patients attending General Hospital Minna (GHM) recorded more prevalence (4.0%) followed by Umaru Sanda Ndayako Hospital Bida (USNHB) with 3.2% and the least (0.6%) was recorded for General Hospital Suleja (GHS). There was significant difference ($P< 0.05$) in incidence of HBV between the hospitals (Table 4.1).

Table 4.1: Seroprevalence of HBV Infection of Patients Attending Some Hospitals in Niger State

Hospitals	NSS	NPS	Prevalence %	Chi square	Significance	Remark
GHS	100	3	0.6	88.360	0.000	*
GHM	100	20	4.0	36.000	0.000	*
GHK	100	12	2.4	57.760	0.000	*
GHL	100	14	2.8	51.840	0.000	*
USNHB	100	16	3.2	46.240	0.000	*
Total	500	65	13.0			

** = significant (P< 0.05) n=100*

Key: GHS - General Hospital Suleja, GHM - General Hospital Minna, GHK – General Hospital Kontagora, GHL - General Hospital Lapai, USNHB -Umaru Sanda Ndayako Hospital Bida, NSS- Number of Samples Screened, NPS- Number of Positive samples, NNS- Number of Negative samples.

4.1.2 Seroprevalence of HBV infection according to gender

The distribution of HBV infection according to gender from the five hospitals revealed that female participants had higher prevalence (6.8%) of HBV infection when compared with their male counterparts with 6.2%, but the difference was not significant ($p \leq 0.05$) ($X^2 = 0.0154$) as shown in Table 4.2. Influence of gender on HBV infection in GHM, GHL and GHK was significant ($p \leq 0.05$) while it was not significant in USNHB ($p>0.05$). In GHS, no incidence of HBV was recorded for males, while three cases were recorded for female patients (n=50).

Table 4.2 Influence of Gender on the Distribution of HBV

Locations	Gender	NSS	NPS	Prevalence (%)	Chi square	Significance	Remark
GHS	Male	50	0	0.0	-		
	Female	50	3	0.6			
GHM	Male	50	12	2.4	0.16	0.026	*
	Female	50	8	1.6			
GHK	Male	50	3	0.6	0.6	0.023	*
	Female	50	9	1.8			
GHL	Male	50	9	1.8	0.228	0.0185	*
	Female	50	5	1.0			
USNHB	Male	50	7	1.4	0.05	0.0597	NS
	Female	50	9	1.8			
Total		500	65	13.0			

$X^2 = 0.0154$ p value ≤ 0.05

Key: NS - Not Significant,*- Significant, GHS - General Hospital Suleja, GHM - General Hospital Minna, GHK – General Hospital Kontagora, GHL - General Hospital Lapai, USNHB -Umaru Sanda Ndayako Hospital Bida, NSS- Number of Samples Screened, NPS- Number of Positive samples, NNS- Number of Negative samples.

4.1.3 Seroprevalence of HBV infection according to age group

The result in Table 4.3 shows the distribution of HBV according to age of patients in all the hospitals under investigation. It was observed that patients within 41- 50 years had higher prevalence of HBV (5.2 %), followed by 18 -30 years with prevalence of 4.2 % while ≥ 50 years recorded a lower prevalence of 1.6%. There was no significant difference in seroprevalence of HBV between age groups 41 – 50 and 18 – 30 years ($p > 0.05$).

Table 4.3 Influence of Age Group on the Distribution of HBV

Age group	NSS	NPS	Prevalence (%)
18 – 30	121	21	4.2
31 – 40	136	10	2.0
41 – 50	202	26	5.2
≥ 50	41	8	1.6
TOTAL	500	65	13.0

$X^2 = 0.4308$ p- value = 0.095

Key: NSS- Number of Samples Screened

NPS- Number of Positive samples.

4.1.4 Distribution of HBV infection based on occupation

The civil servants had more prevalence of 6.4 % followed by house wives (3.2 %) and the least was observed for participants who were students (1.6 %). The results obtained were used to correlate HBV infection with risk factors at 95 % confidence interval and a p value ≤ 0.05 was considered significant (Table 4.4). There was no significant difference ($p > 0.05$) in the influence of occupation or status on seroprevalence of HBV.

Table 4.4: Influence of Occupation on the Distribution of HBV

Occupation	NSS	NPS	Prevalence (%)
Civil servants	247	32	6.4
House wives	131	16	3.2
Students	50	6	1.6
Others	72	9	1.8
Total	500	65	13

$X^2 = 0.059$; $p = 0.5$

Key: NSS- Number of Samples Screened, NPS- Number of Positive samples.

4.1.5 Influence of blood transfusion on the distribution of HBV

Patients without history of blood transfusion recorded high prevalence (7.8%) compared to those with history of blood transfusion (5.2%). The results obtained were used to correlate HBV infection with risk factor at 95% confidence interval and a p value ≤ 0.05 was considered significant (Table 4.5). The influence of blood transfusion on HBV prevalence in this study was not significant ($p > 0.05$)

Table 4.5: Influence of Blood Transfusion on the Distribution of HBV

Blood transfusion	**NSS**	**NPS**	**Prevalence (%)**
Yes	169	26	5.2
No	331	39	7.8
Total	500	65	13

$X^2 = 0.154$ p- value = 0. 568

Key NSS- Number of Samples Screened

NPS- Number of Positive samples

4.1.6: Seroprevalence of HBV infection according to marital status

The result in Table 4.6 shows the distribution of HBV according to marital status of patients in all the hospitals under investigation. It was observed that unmarried patients had higher prevalence of 1.33% compared to married patients with 1.27%. There was no significant difference in the influence of marital status on HBV prevalence amongst patients ($p > 0.05$).

Table 4.6 Influence of Marital Status on the Distribution of HBV

Marital status	**NSS**	**NPS**	**Prevalence (%)**	**Significance**	**Remark**
Married	450	57	1.27		
Single	50	8	1.33	0.0971	NS
Total	500	65			
$X^2 = 0.000$		$P < 0.05$	n =500		

Key: NS = Not Significant

NSS- Number of Samples Screened

NPS- Number of Positive samples

4.1.7 Influence of sharing of razor blade/ utensils on the distribution of HBV

Figure 4.1 shows the influence of sharing of razor blade and utensils on the prevalence of HBV amongst patients. Individuals who shared razor blades and utensils with HBV positive individuals were 2.48 times more likely to test positive to HBV in GHK, GHL, and USNHB, than those in GHM and GHS. The influence of this factor was significant ($P < 0.05$) when GHK, GHL, and USNHB are compared to GHM and GHS; but the influence was not significant ($p > 0.05$) between GHK, GHL, and USNHB. Logit model $P<0.05$. $X^2(12) = 8.876$, $P= 0.714$. The model explained 3.3 % (Nagelkerke R^2) of the variance in HBV status and correctly classified 87% of cases as shown on figure 4.1 below.

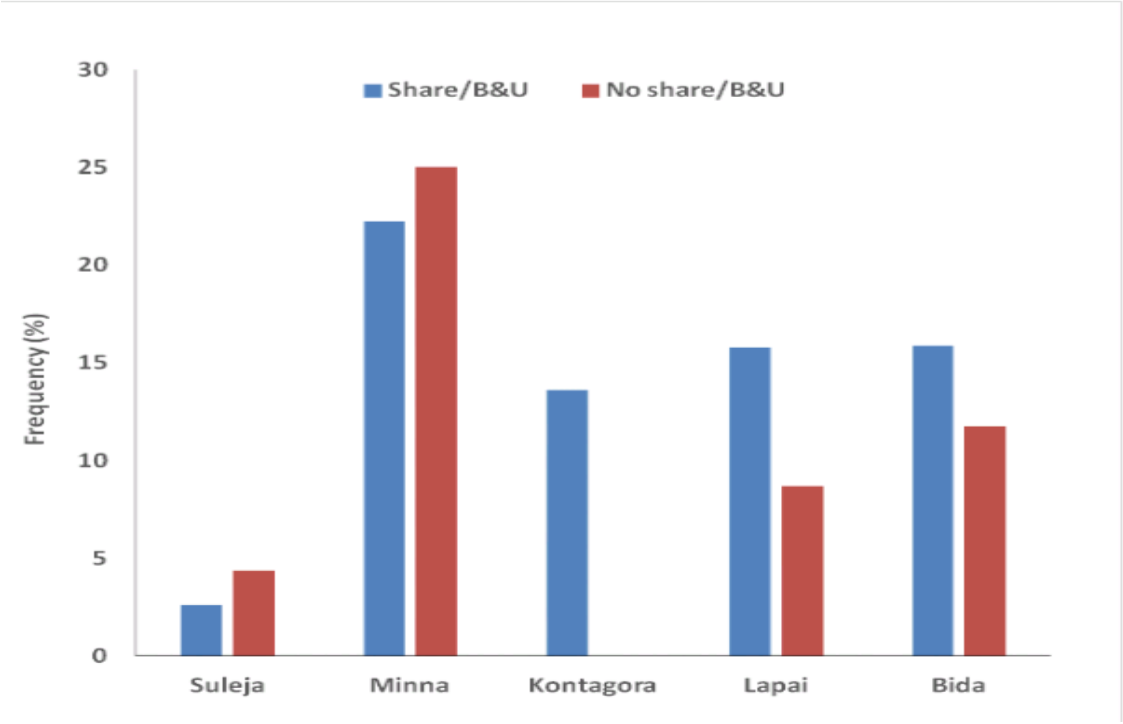

Figure 4.1: Influence of Sharing of Razor Blade/ Utensils on the distribution of HBV.

4.1.8 Influence of vaccination on the distribution of HBV

The distribution of HBV based on the history of vaccination did not contribute significantly to the prevalence of HBV in the study area. The logit model was not statistically significant at $P<0.05$. $X^2(12) = 8.876$, $P= 0.714$. The model explained 3.3 % (Nagelkerke R^2) of the variance in HBV status and correctly classified 87% of cases as shown on figure 4.2 below.

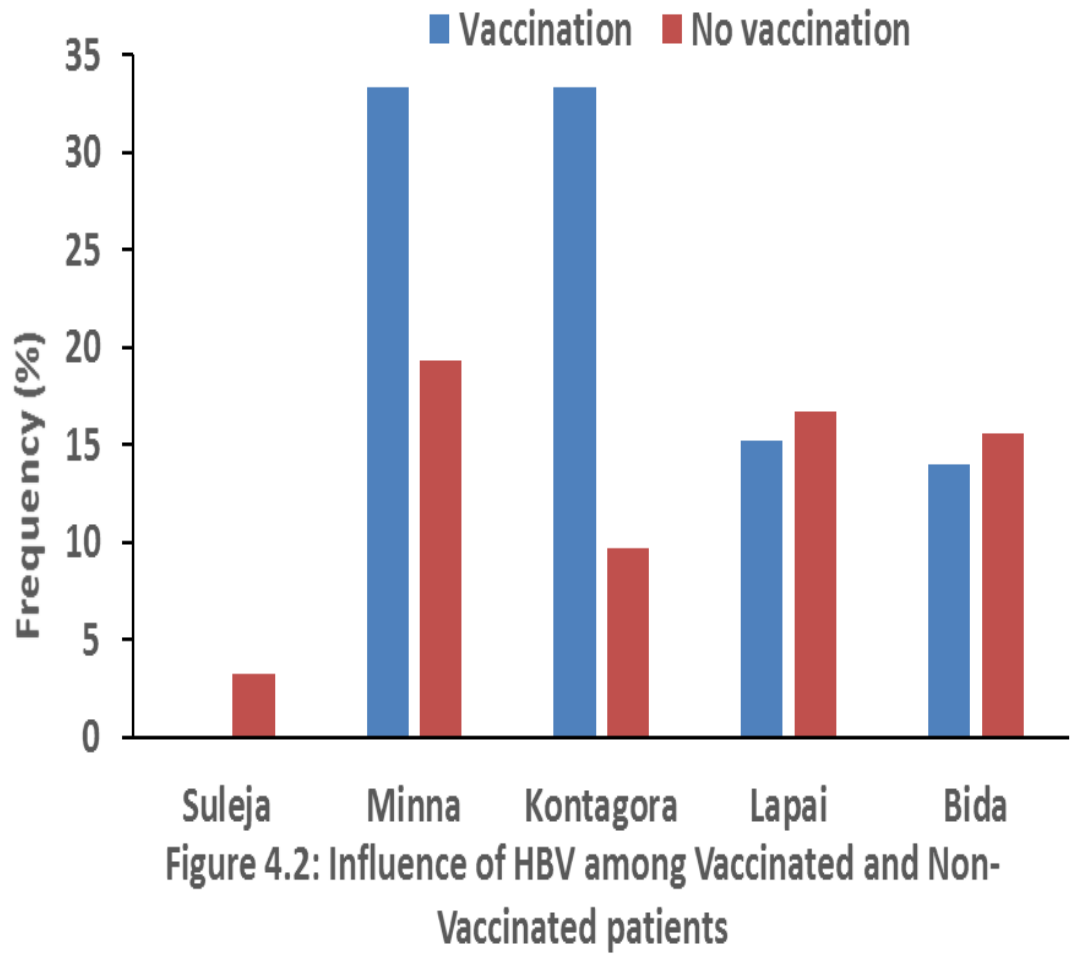

Figure 4.2: Influence of HBV among Vaccinated and Non-Vaccinated patients

4.1.9: Distribution of HBV Genotypes in the study population based on location

Plates I, II, III & IV in Appendix D show the amplicons obtained from the DNA of HBV isolated from positive samples. Out of the 65 HBV positive samples obtained, 32 (49.2%) were genotyped successfully. Among the 32 samples studied, 41 HBV genotypes were identified as follows: genotype A: 14 (34.1%) with 68bp; genotype C: 4 (9.8%) with 122bp; genotype D: 1 (2.4%) with 119bp; genotype E: 19 (46.3%) with 167bp; genotype F: 3 (7.3%) with 122bp; and 15 (46.9%) patients had mixed genotypes (Table 4.6).

Table 4.7: Distribution of HBV Genotypes in the Study Population Based on Location

Locations	Genotype A	Genotype B	Genotype C	Genotype D	Genotype E	Genotype F
GHS	-	-	1	1	-	1
GHM	3	-	-	-	4	-
GHK	3	-	1	-	4	1
GHL	3	-	-	-	5	-
USNHB	5	-	2	-	6	1
Total	14(34.1%)	-	4(9.8%)	1(2.4%)	19(46.3%)	3 (7.3%)

Key: GHS - General Hospital Suleja, GHM - General Hospital Minna, GHK – General Hospital Kontagora, GHL - General Hospital Lapai, USNHB -Umaru Sanda Ndayako Hospital Bida.

4.1.10 Distribution of mixed Genotypes in the study population

Table 4.7 represents the distribution of the occurrence of mixed genotypes amongst the study population. It shows that for mixed genotype A/E had a significantly higher ($p < 0.05$) occurrence of 10 (31.25%) compared to mixed genotype C/E with occurrence of 3(9.375%) and A/F with occurrence of 1(1/32). The occurrence of mixed genotypes A/E spread across GHM, GHK, GHL and USNHB with USNHB recording the highest number. Mixed genotypes C/E was only recorded in GHK, while mixed genotypes A/F was recorded only in USNHB.

Table 4.8: Distribution of Mixed Genotypes in the Study Population

Locations	Genotypes A/E	Genotypes C/E	Genotypes A/F
GHS	-	-	-
GHM	2	-	-
GHK	1	3	1
GHL	3	-	-
USNHB	4	-	
Total	10 (31.25%)	3 (9.375%)	1 (3.125%)

Key: GHS - General Hospital Suleja, GHM - General Hospital Minna, GHK – General Hospital Kontagora, GHL - General Hospital Lapai, USNHB -Umaru Sanda Ndayako Hospital Bida.

4.1.11. Alignment of DNA Sequences

The 5 samples sequenced were BLAST under N option. Information obtained from the queried sequences and the closest corresponding outcomes from the GenBank revealed the result shown in Figure 4.3. The results indicated that the queried samples had genotypes E with percentage identity ranging from 98-100 (Table 4.9).

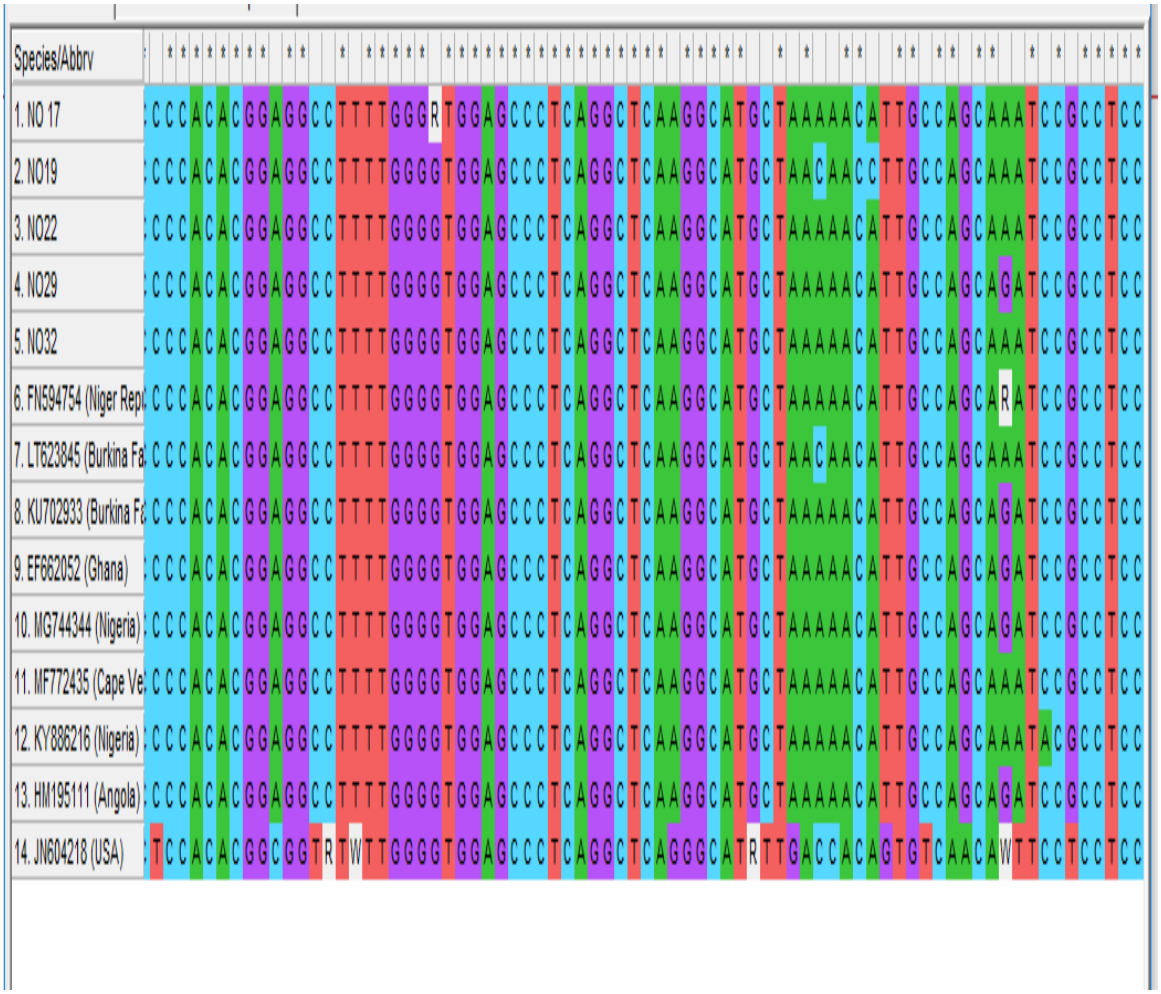

Figure 4.3: Extract of Allignment from Sequences of Five Samples and Nine Reference Sequences Retrieved from the Genbank.

Table 4.9: Blast N Pairwise Alignment of Five Amplicon Sequences

Sample	Source	Length	Score	E-value	Identities	Reference strain	Inference
17	HBV S gene for S protein, strain BFA16-H-83.	3212	223	2E-54	98	LT623848.1	genotype E
19	HBV complete genome, strain 42349	3212	223	2E-54	98	LT62347.1	genotype E
22	HBV S gene for S protein, strain BFA16-H-83.	3212	226	2E-55	98	FM545841.1	genotype E
29	HBV isolate BF337 large S protein (S) gene, complete cds	3200	226	2e- 55	100	FN545827.1	genotype E
32	Hepatitis B virus S gene for S protein, strain BFA16-H-101	3212	228	5E-56	99	LT623848.1	genotype E

*The query sequences cover 100%

4.1.12. Phylogenic Tree of HBV Strains in the samples

The tree revealed that DNA Sample 17 is closely related to KU702933 (Burkina Faso) and KY886216 (Nigeria); Sample 19 is related to LT623845 (Burkina Faso) and JN604218 (USA); Sample 22 is related to FN594754 (Niger Republic); Sample 29 is related to MF772435 (Cape Verde); while Sample 32 is related to three (3) different strains EF662052 (Ghana), MG744344 (Nigeria), and HM195111 (Angola) as shown in Figure 4.4 below.

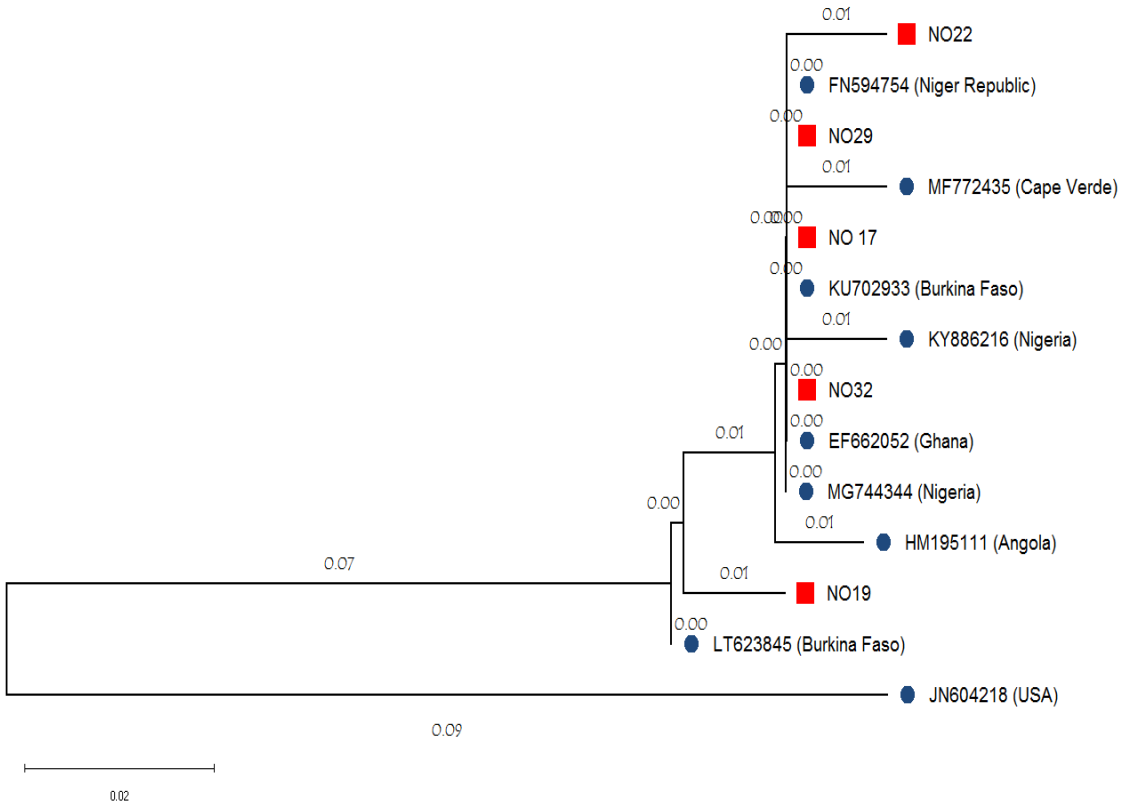

Figure 4.4: Phylogenetic Tree of HBV strains.

4.2 Discussion

4.2.1 Seroprevalence of HBV infection of patients attending some Hospitals in Niger State

Despite the availability of relatively effective vaccines against HBV and the progress that has been made in antiviral treatments, hepatitis B continues to be a major public health problem in the world (WHO, 2000).

The present study determined the prevalence, distribution, factors associated with infection and identifying the circulating genotypes of Hepatitis B virus amongst patients attending some hospitals in Niger State. The study is the first to determine sero-prevalence of HBV amongst the general population attending some hospitals in Niger State on the basis of age, gender, occupation, marital status, vaccination, and blood transfusion. The study established 13% HBV prevalence that indicates high endemicity of HBV in the study area. A number of studies have reported percentage prevalence of HBV amongst different populations in Nigeria. In a report by Adabara *et al*. (2012), 6.5% prevalence of Hepatitis B virus among women attending antenatal Clinic, General Hospital, Minna, Niger state was observed, while a study by Kuta *et al*. (2014) on Sero-prevalence of Hepatitis B Virus Surface Antigen infection among pregnant women visiting Garki Hospital, Abuja, Nigeria for Antenatal care, reported 10% prevalence. The two studies were centered on women attending ante-natal clinics, while the present study cut across different groups attending some hospitals.

In a National Survey undertaken by Adebola *et al*. (2016) on Sero-prevalence of Hepatitis B Infection in Nigeria, 12.2% prevalence was reported. A review article on the same subject matter reported a prevalence rate of 4.3 – 23.3% (Berinyuy *et al*., 2019). It

is pertinent to note that our study is different from those reported above because it is the first to focus on the general population of patients (males and females of varying ages, marital status, history of vaccination, sharing of utensils and occupation) attending some hospitals within a period, from different zones of Niger State. However, the cumulative percentage prevalence of 13% reported in our study falls within the ranges reported in all the reports cited above. It might have indicated a stable prevalence, but the need for continuous monitoring of HBV prevalence amongst our growing population cannot be overemphasized because Nigeria with an estimated population of 190 million people has hepatitis B prevalence of 8.1% (WHO, 2017).

4.2.2 Influence of Gender on the distribution of HBV

Female participants had higher prevalence of 6.8% HBV infection compared to their male (6.2%) counterparts. This finding disagrees with other previous studies (Doumbia *et al*., 2013; Saeeda, 2009; Al-Suraifi *et al*., 2016; Ahmed *et al*., 2020) where higher prevalence of HBV was observed in men. In spite of the observed differences in infection rate between males and females, sex is not a significant factor influencing HBV infection in the study area.

Furthermore, the high positivity rate (2.4 and 1.8%) observed for the male gender in Minna and Lapai hospitals respectively may not be unconnected with the fact that high risk behaviors like sharing of barber clippers nail cutters, tribal mark scalpel, violence and conflicts in which blood contact may occur are associated with male folklore in these study areas. It may also be unconnected to the higher exposure to occupational HBV risk factors in men.

4.2.3 Influence of Age group on the distribution of HBV

The results obtained further revealed that the age groups; 41 - 50 and 18 - 30 years had highest prevalence of 5.2 and 4.2% HBV infection respectively. This could be attributed to the fact that these groups are supposedly sexually active. In countries where adult horizontal transmission patterns are the principal transmission routes, the incidence of HBV infection is highest in adults (Custer *et al*., 2004). Our study also revealed that ≥ 50 years recorded a lower prevalence of 1.6%. This finding agrees with World Health Organization report that the low prevalence of individuals in the age group ≥ 50 years could indicate that several people in this group might have died from cirrhosis or liver cancer due to lack of medical care (WHO, 2015). In an earlier study in Iran., the highest prevalence of HBV was observed in the 50-59 age group (Zali *et al*., 1996).

4.2.4 Influence of occupation on the distribution of HBV

Civil servants recorded the highest prevalence of 6.4% followed by house wives with 3.2% and the least was observed for students sampled in this study. The effect of occupation on prevalence of HBV was observed to be significant with civil servants accounting for a higher prevalence possibly due to exposure in their places of work. In a similar study conducted to determine the impact of economic and social factors on the prevalence of hepatitis B in Turkey, it was observed that welfare indicators such as number of bedrooms and occupational status of households were statistically significant at 1% significance level and negatively correlated with the probability of having HBV (Tosun *et al*., 2018).

4.2.5 Influence of blood transfusion on the distribution of HBV

The study further revealed that patients without history of blood transfusion recorded higher prevalence (7.8%) compared to those with history of blood transfusion (5.2%). Blood transfusion is also a common source of HBV transmission in countries where the blood supply is not screened for HBsAg. The impact of blood transfusion as a risk factor for HBV infection was significant. Over the past decades, the risk of HBV transfusion-transmission has been steadily reduced through measures like selection of donors based on risk-behavior evaluation, development of increasingly more sensitive HBs-Ag assays and recruitment of volunteer donors (Candotti *et al.*, 2017), but these measures are not readily available in our localities where blood transfusion may not be subjected to these preventive measures.

4.2.6 Influence of marital status on the distribution of HBV

The study further revealed single patients had high prevalence of 1.33% than married patients with 1.27%. Ugwuja, and Ugwu, (2010) reported 15.52% prevalence of HBV for married people against 14.95% for singles. The study revealed that there was no significant difference in the HBV status of married and single patients, therefore, marriage is not a predisposing factor influencing HBV infection in the study area. In the study conducted by Zali *et al*., 1996, history of blood transfusion was not a major risk factor which corresponds to our study, but factors like sexual relationship, injections, old age, masculinity, marital status, history of contact with hepatitis were considered risk factors.

4.2.7 Influence of sharing of razor blade/utensils on the distribution of HBV

Sharing of razor blade/Utensils contributed significantly to the distribution of HBV in the study areas. Individuals who shared razor blades and utensils with HBV positive

individuals were 2.48 times more likely to test positive to HBV than those who did not share razor blades/ utensils. In a similar study reported by WHO, (2001), the virus may spread from inanimate objects such as shared towels or toothbrushes, because it can survive for at least 7 days outside the body and can be found in high titers on objects, even in the absence of visible blood.

Sharing of objects, the re-use of contaminated equipment for medical, cosmetic or dental procedures, failure to use appropriate disinfection and sterilization practices for equipment and environmental surfaces, and improper use of multidose medication vials, can result in the transmission of HBV in many developing countries (Kann *et al*., 2007; Simonsen *et al*., 1999).

4.2.8 Influence of vaccination on the distribution of HBV

The study further revealed that history of vaccination does not contribute significantly to the prevalence of HBV in the study area, although vaccine is 80-100% effective in the prevention of HBV infection (Schillie *et al.,* 2017). Furthermore, the combination of vaccine plus hepatitis B immunoglobulin is superior to vaccine alone (Schillie *et al.,* 2017; Araujo, 2015). In 10- to 22-year follow-up studies there were no cases of hepatitis B among those with a normal immune system who were vaccinated (WHO, 2014).

4.2.9 Distribution of HBV Genotypes in the study population based on location

In the genotypic diversity study, five (5) HBV genotypes (A, C, D, E and F) were identified. Our result corroborates an earlier report that the five genotypes above are those in circulation in Africa (Zampino *et al*., 2015).

Genotype E was the most prevalent (46.3%) while genotype A was the second most prevalent. These observations are in agreement with what was reported by Odemuyiwa

et al., (2001); Forbi *et al*., (2010); Faleye *et al*., (2015); and Ahmed *et al*., (2020). They all reported that genotype E is the most prevalent genotype in Nigeria, followed by genotype A.

4.2.10 Distribution of mixed Genotypes in the study population

Our study has established the existence of mixed or co-infection of genotypes in some positive individuals. It is interesting to note that despite the identification of some positive samples with co-infection of two different genotypes, HBV A/E was the most prevalent, followed by HBV C/E. It is pertinent to note that our study has identified the major genotypic contributors to the HBV burden in Niger state, thus providing cogent information that would assist in the design of HBV cell culture and animal models to test novel treatment strategies and vaccines. In addition, the present study provides an up-to-date analysis of HBV genotypic data in Niger State that will form an initial basis for estimating how genotypes contribute to the burden of chronic HBV infection in Niger State and Nigeria at large.

Individuals with co- infection are likely to encounter the challenges of treatment failures and drug resistance because a single drug of treatment may not possess the capacity to destroy two variants of the virus in the same individual at the same time. Therefore, information emanating from this study will go a long way in assisting the design of treatment protocols for different categories of infected patients based on the identified genotypes.

4.2.11 Blast N Pairwise Alignment and Phylogenetic tree of HBV strains

The alignment of sequences was the bases for the construction of the phylogenic tree as shown on figure 4.4 The tree topology formed by the five DNA samples showed a probable association with genotypes originating from some West African countries like Niger Republic and Ghana that are close-door neighbors to Nigeria and Burkina Faso.

In addition, a probable association was observed with genotypes from Angola, Southern African country and United States of America (USA). The most probable circulation place for genotypes A and E are Burkina Faso, Nigeria, Niger Republic, and USA; while genotype C has its circulation point only in Niger Republic. The identities of the genotypes for samples 29 and 32 could not be ascertained from sequencing but based on the ascension numbers used; sample 29 has a probable association with a genotype originating from Cape Verde, while sample 32 has a probable association with a genotype originating from Ghana, Nigeria, and Angola. The observed trans-national association of genotypes in Niger State is probably due to trans-border interactions between citizens of the different countries through trade, tourism, and marriage.

4.2.13 Trouble shooting

Out of the 65 positive samples, only 32 were successfully genotyped. In a multiplex PCR, it is possible for one locus to become over loaded before another locus achieves enough concentration to be identified in electrophoresis. From the 32 samples that were successfully genotyped, only five were viable for sequencing which could be attributed to the annealing temperature which depends primarily on length and base composition of the PCR primer. Increasing the annealing, temperatures decreases the chances of unspecific PCR products. Multiple interacting factors such as mixed trace signals (multiple picks), short/poor quality trace and noisy trace where the bands become difficult to read (Sanger *et al*., 2002).

In order to prevent the loss of samples in future research, it is recommended that PCR reaction is performed for each primer pair independently for at least 1-4 samples and submit these samples in a dilution series to determine whether a common dilution will work for these loci. If a common dilution can be determined, then the multiplex PCR

can be performed with all the primer pairs. It is better to ensure that all primer concentrations are the same and the annealing temperature need to be considered in such a way that it is lower than the melting temperatures of the primers by at least a magnitude of 2. It may be that some primer pairs can be multiplexed together while others need to be amplified independently before combining to submit for further analysis.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study has revealed a 13.0% prevalence of HBV infection in Niger state. It has also established that gender, age, history of blood transfusion, and occupation were predisposing factors to HBV infection in the study areas. Hepatitis B virus genotypes E and A are the predominant genotypes in the State. The study also observed that A/E HBV co-infection was common in Niger State. The sequencing and phylogenetic analysis of the observed genotypes conforms with the hypothesis of worldwide distribution of genotypes A and E.

5.2 Recommendations

i. There is the need for more awareness and sensitization of people on the prevalence of HBV infection in Niger State. People need to be enlightened on the mode of transmission and spread of the virus through different information media outfits.

ii. HBV infection should be regarded as a serious blood borne viral pathogen and should be tested for before blood transfusion and marriage solemnization are carried out.

iii. The health care system should be empowered by Government and non-governmental organizations to carter for diagnosis and support of infected individuals.

iv. Diagnosis of patients with HBV infection should be undertaken to the level of identifying the genotype to enable target specific medication/ treatment.

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APPENDIX A

NIGER STATE HOSPITALS MANAGEMENT BOARD
GENERAL HOSPITAL MINNA

ADDRESS:
No. 1 Hospital Road,
P.M.B. 2 Minna
Niger State, Nigeria.
Tel: 08109999223

E-Mail: Genhospminna@yahoo.com
Genhospminna@gmail.com

1st August, 2017
HMB/GHM/136/VOL.III/458

The Dean,
Department of Microbiology,
Federal University of Technology,
Minna.

Through:
Adamu Aishatu,
Department of Microbiology,
Federal University of Technology,
Minna.

Sir,

RE: Adamu Aishatu (PhD/SLS/2015/817)
RESEARCH, ETHICS AND PUBLICATION COMMITTEE'S
APPROVAL TO CONDUCT RESEARCH.

The bearer, a student of your department sought for permission to conduct research on "**SERO PREVALENCE AND MOLECULAR CHARACTERIZATION OF HEPATITIS B VIRUS ISOLATED FROM PATIENTS ATTENDING SELECTED HOSPITALS IN NIGER STATE**"

The committee after going through her proposal has given her the approval to conduct the Research.

A copy of her final findings must be submitted to the committee as a pre-requisite to this approval.

Thank you for your cooperation.

Pharm. Ibrahim Shehu Madara
B.Pharm, MPharm.
Secretary REPC
For: **Medical Director**

MINISTRY OF HEALTH
NIGER STATE GOVERNMENT OF NIGERIA

Block 'C' First Floor, Abdul-Kareem Lafene Secretariat Complex, Paiko Road, P.M.B. 57, Minna, Niger State.
e-mail: ngsmohmx@yahoo.com

MOH/STA/95/I/V-I

15th December, 2016

Adamu Aishatu,
Department of Micro-Biology,
School of Life Sciences,
Federal University of Technology,
Minna.

ETHICAL APPROVAL

The Niger State Ministry of Health has given approval for the implementation of your research protocol titled:
"Sero prevalence and Molecular characterization of Hepatitis B virus amongst women receiving medication in selected hospitals in Niger State". (General Hospital Minna, General Hospital Kontagora, Etsu Umaru Sanda Ndayako Hospital Bida, General Hospital Suleja, General Hospital Lapai, and Rural Hospital Agaie, Rural Hospital Kagara, Rural Hospital Kutigi, Rural Hospital Sabon Wuse, and Rural Hospital Wushishi) respectively.

You are required to submit periodically, a review of the study to this committee. On completion of the study, a final full review must be submitted to this committee. Any adverse effect in the course of the studies must be brought to the notice of the committee within Seven (7) days. This committee must be inform before your research findings are published

Thank you.

Dr. Abdullahi U. Imam
Director Medical Services & Training
Chairman Research Ethnics Committee

Appendix B

QUESTIONNAIRE ON PHD RESEARCH WORK FOR BIODATA AND INFORMATION OF PATIENTS

Identification code _____

Age_____

Gender Male ☐ Female ☐

Occupation: Full Housewife ☐ Civil Servant ☐ others ☐

Any history of HBV Vaccinations Yes ☐ No ☐

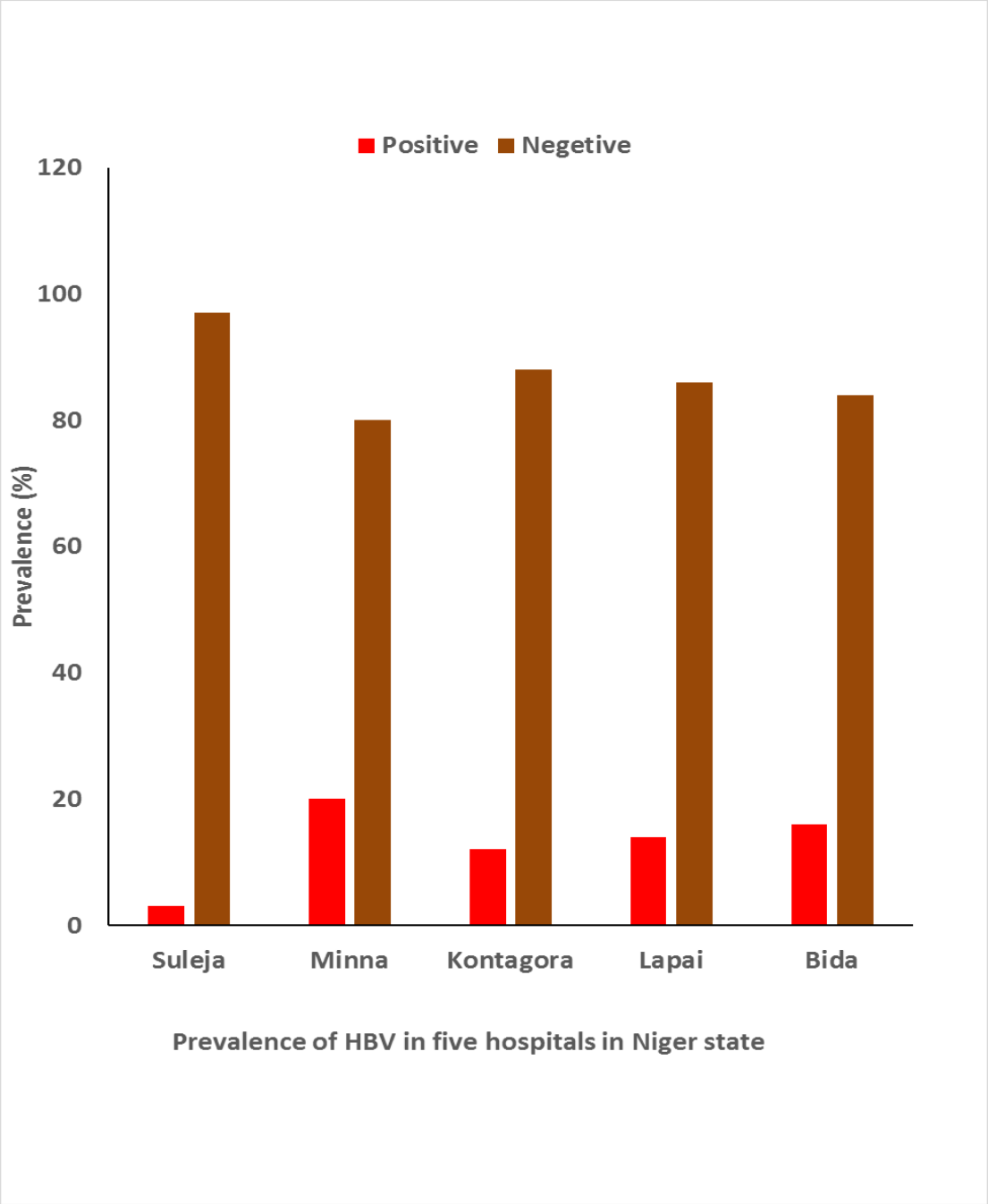
History of blood transfusion Yes ☐ No ☐

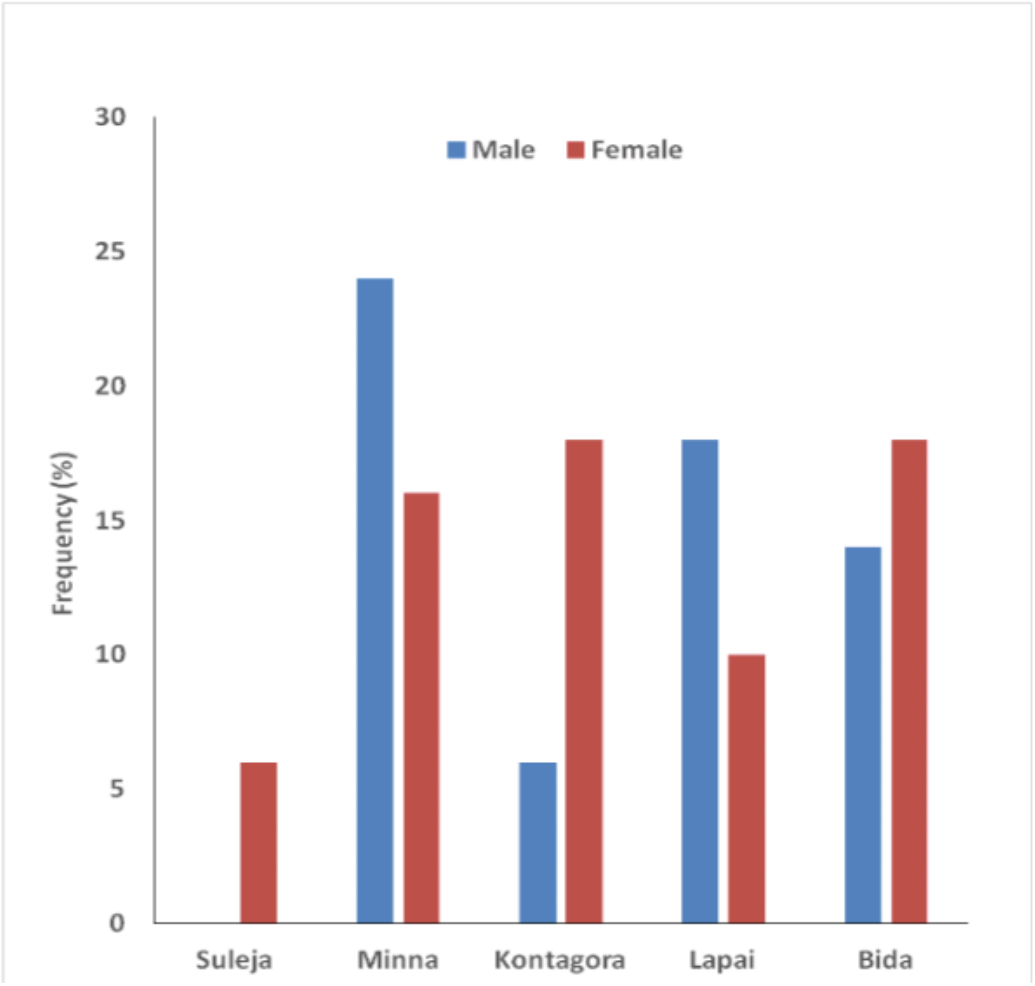
Marital Status Married ☐ Single ☐

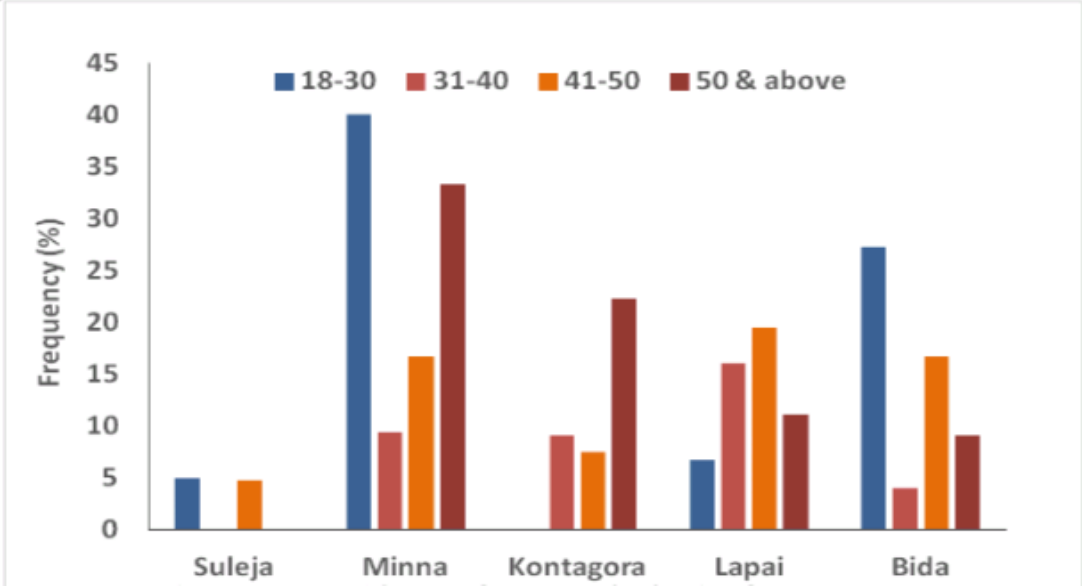
Sharing of blade and utensils Yes ☐ No ☐

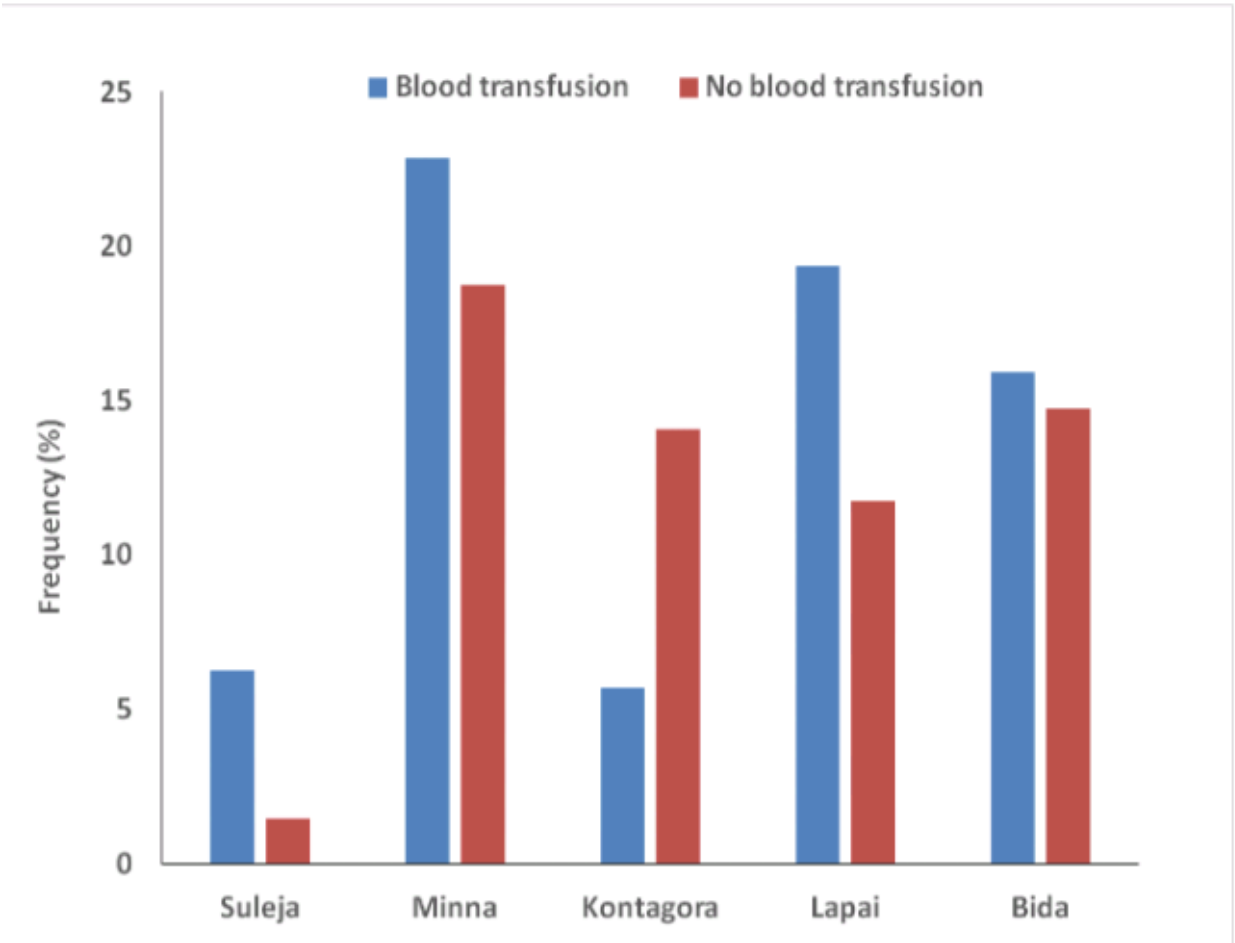
Appendix C

Bar charts of distribution of HBV infection based on location and other parameters




30
25
20
15
10
5
0
Frequency (%)
Male
Female
Suleja
Minna
Kontagora
Lapai
Bida


18-30
31-40
41-50
50 & above
Frequency (%)
45
40
35
30
25
20
15
10
5
0
Suleja
Minna
Kontagora
Lapai
Bida


Blood transfusion
No blood transfusion
Frequency (%)
25
20
15
10
5
0
Suleja
Minna
Kontagora
Lapai
Bida

Appendix D

Amplicons obtained from the DNA of some positive HBV samples

Plates 1, 2, 3 & 4 represent the amplicons obtained from the DNA isolates of HBV positive samples. The 5'UTR of the genome region with different sizes of molecular weight markers were amplified using the Nested PCR.

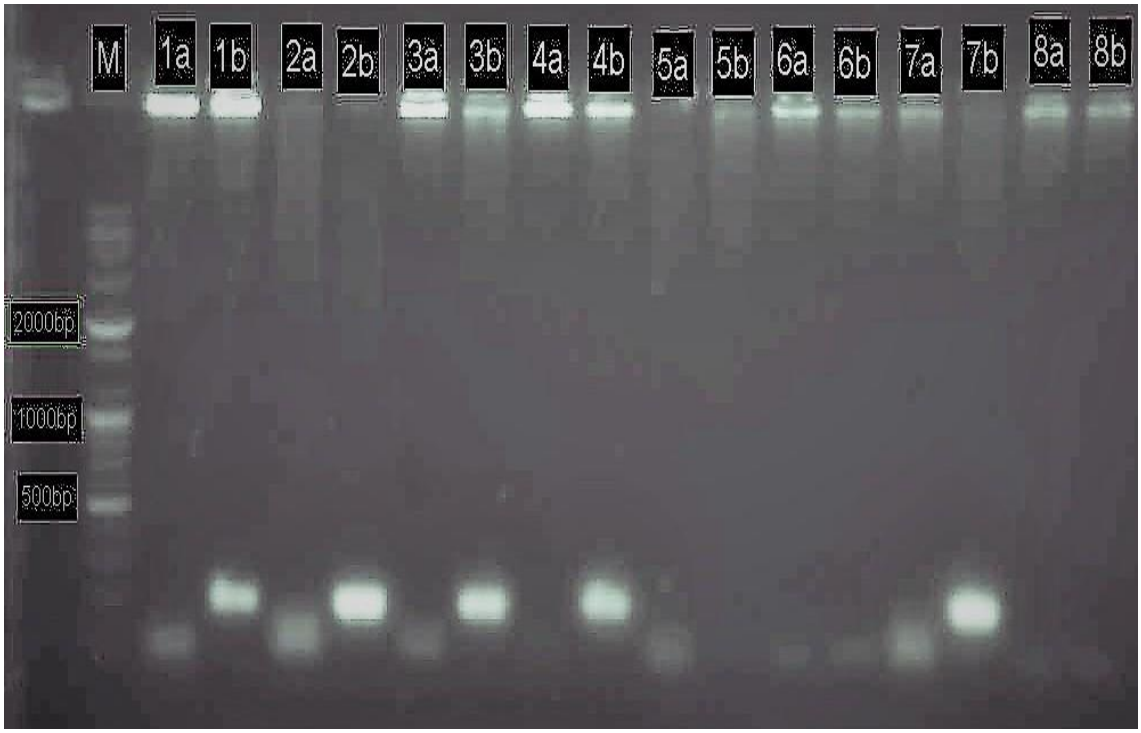


Plate I: Detection of HBV DNA by Gel Electrophoresis of PCR products using Mix A and Mix B Primers Lane 1-8 are PCR products. M indicates molecular weight marker and DNA ladder.

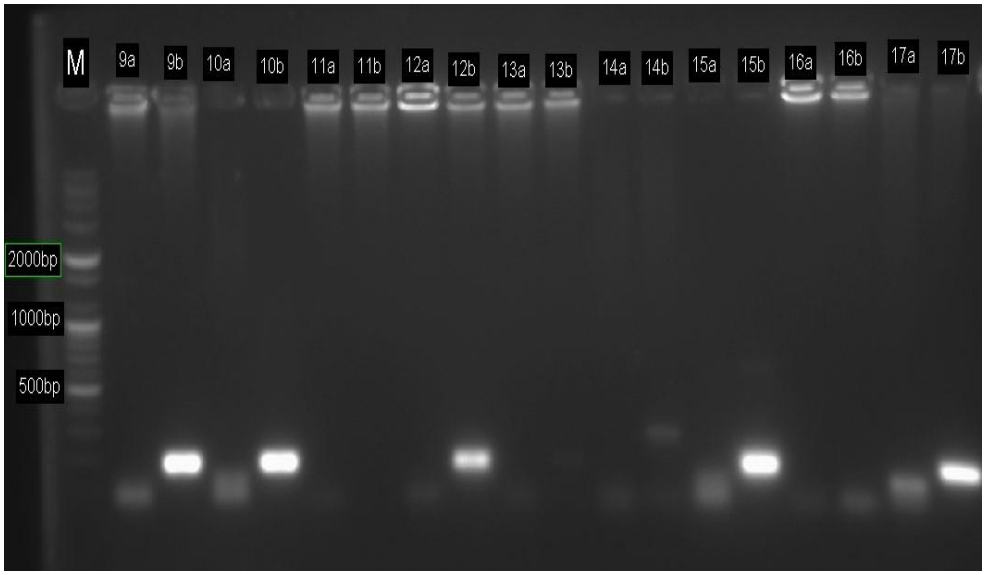


Plate II: Detection of HBV DNA by Gel Electrophoresis of PCR products using Mix A and Mix B Primers Lane 9-17 are PCR products. M indicates molecular weight marker and DNA ladder.

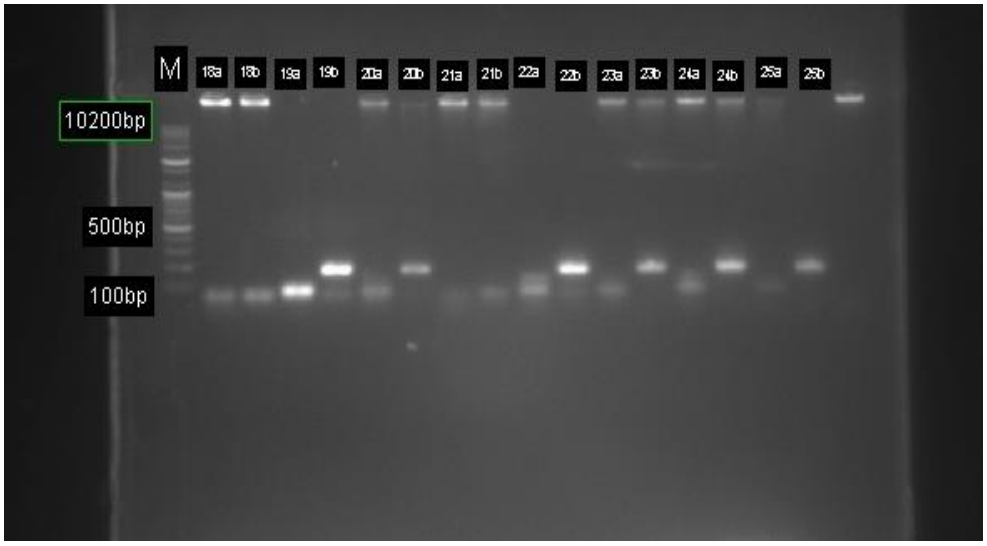


Plate III: Detection of HBV DNA by Gel Electrophoresis of PCR products using Mix A and Mix B Primers Lane 18-25 are PCR products. M indicates molecular weight marker and DNA ladder.

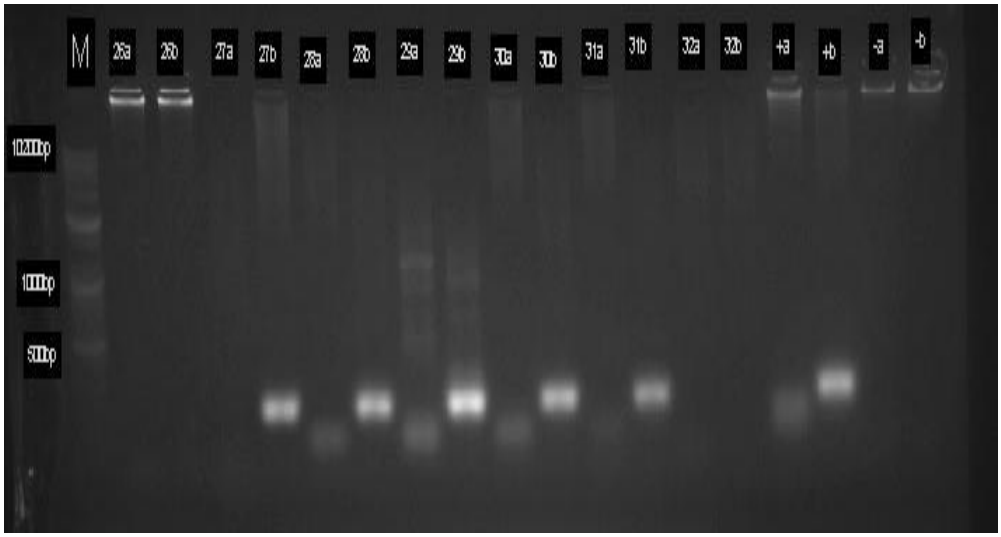
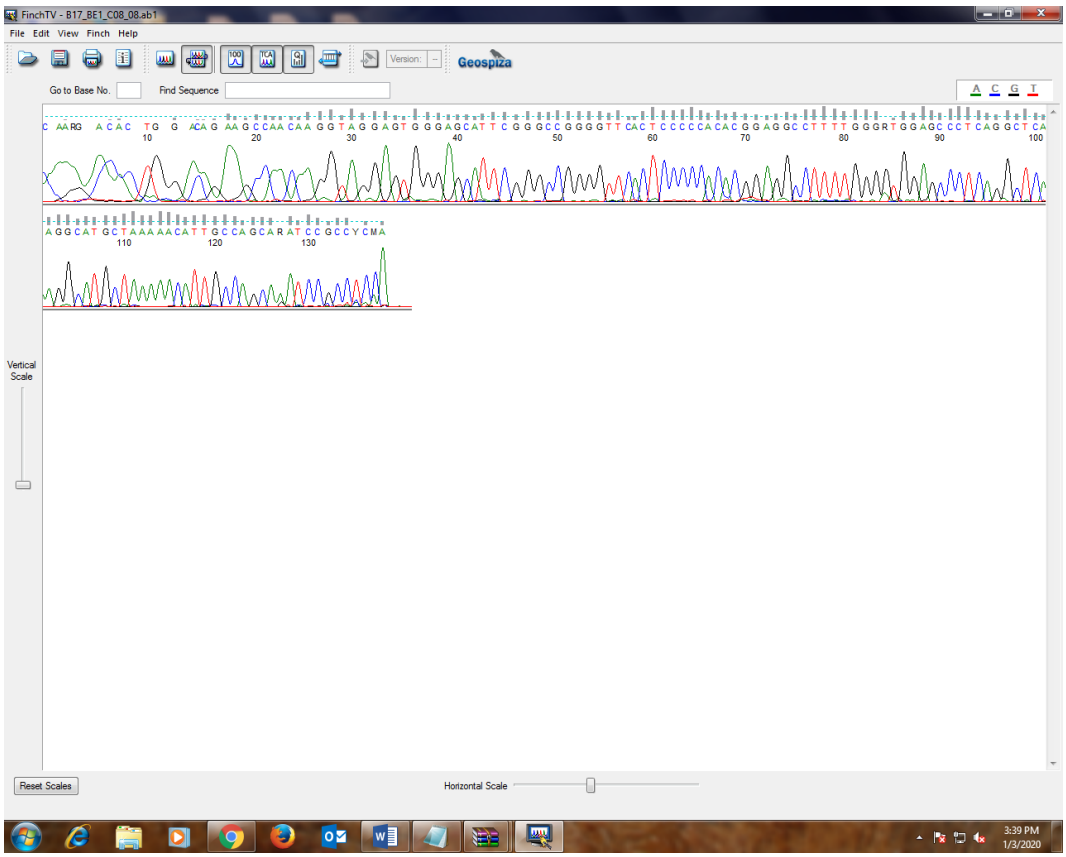


Plate IV: Detection of HBV DNA by Gel Electrophoresis of PCR products using Mix A and Mix B Primers Lane 26-32 are PCR products. M indicates molecular weight marker and DNA ladder.

Appendix E

Micrographs of five (5) positive samples that were finally sequenced

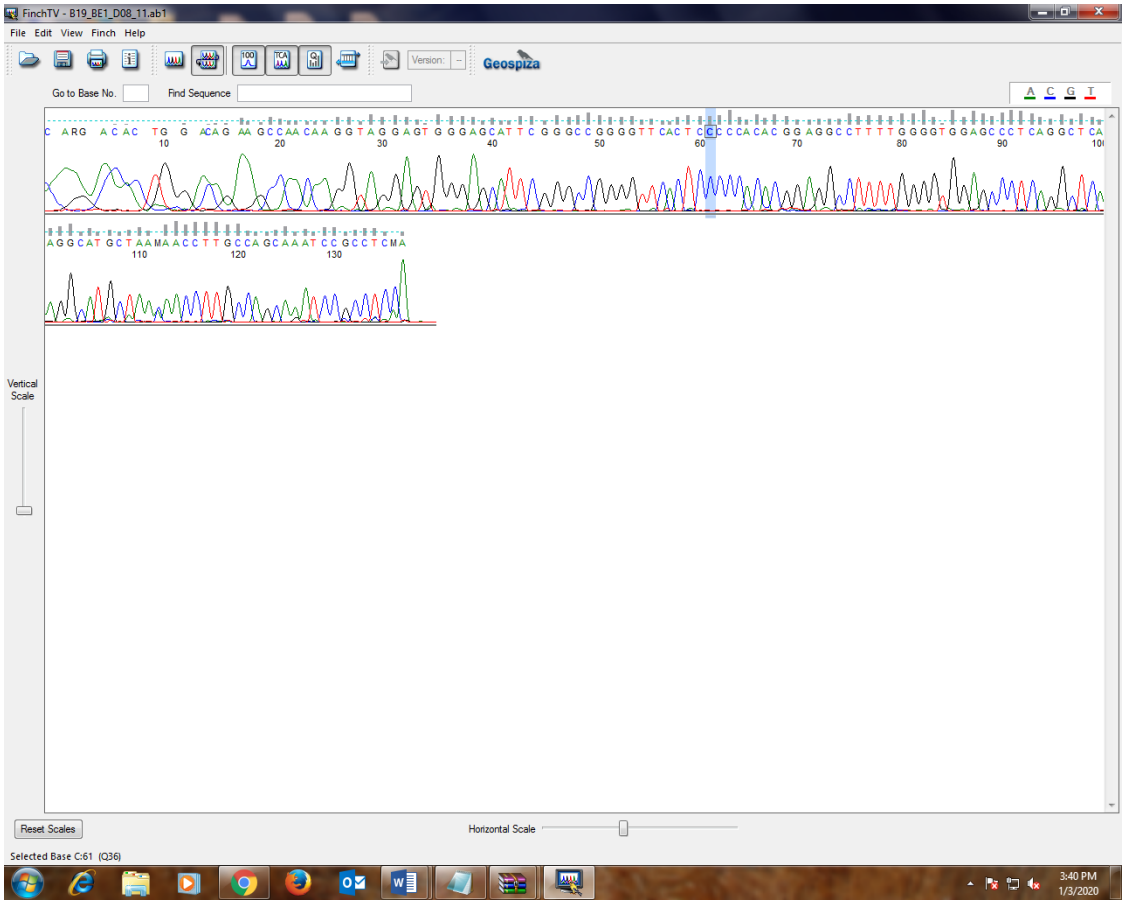


Sample No. 17

GGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTC

CCCCACACGGAGGCCTTTTGGGRTGGAGCCCTCAGGCTCAAGGCATGCTAA

AAACATTGCCAGCAAATCCGCCTCCA

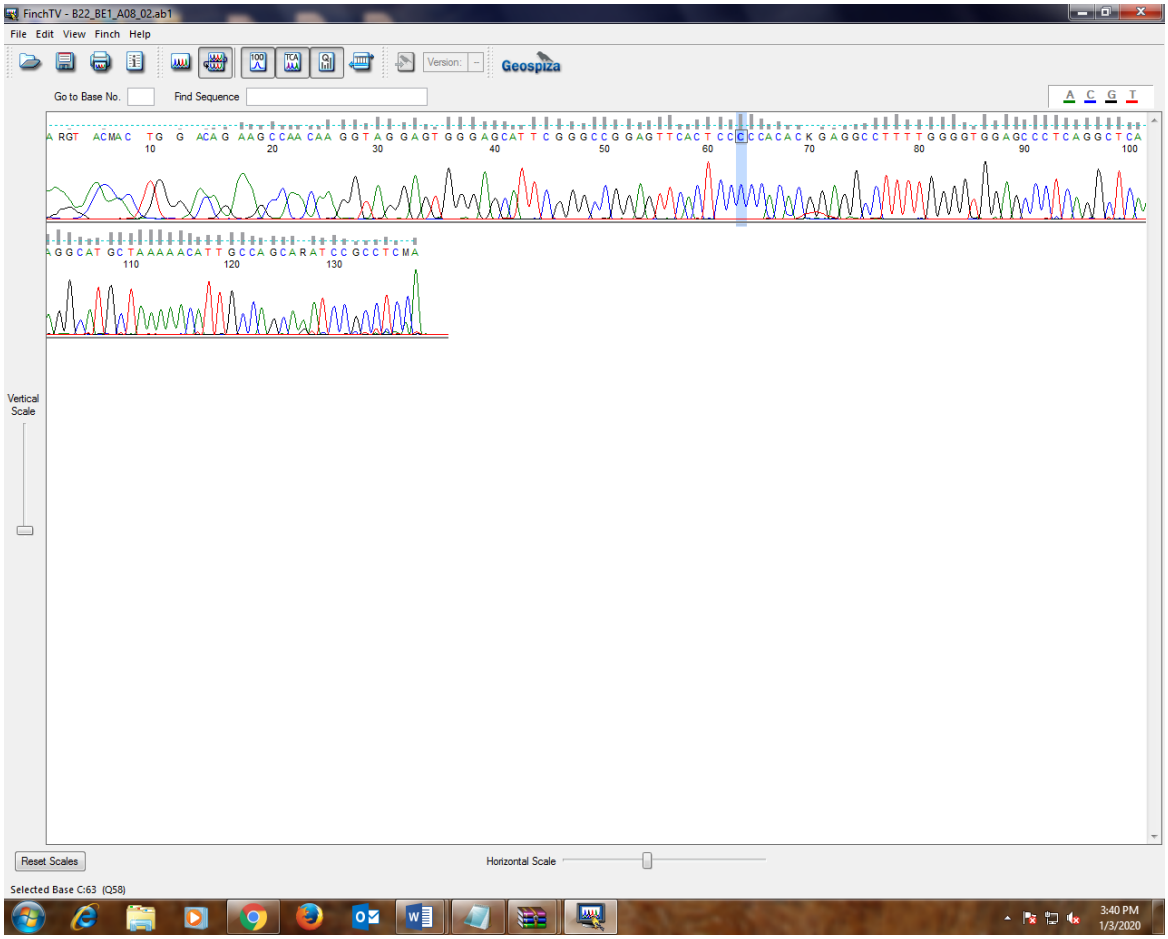


Sample No. 19

TGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACT

CCCCCACACGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTA

ACAACCTTGCCAGCAAATCCGCCTCCA

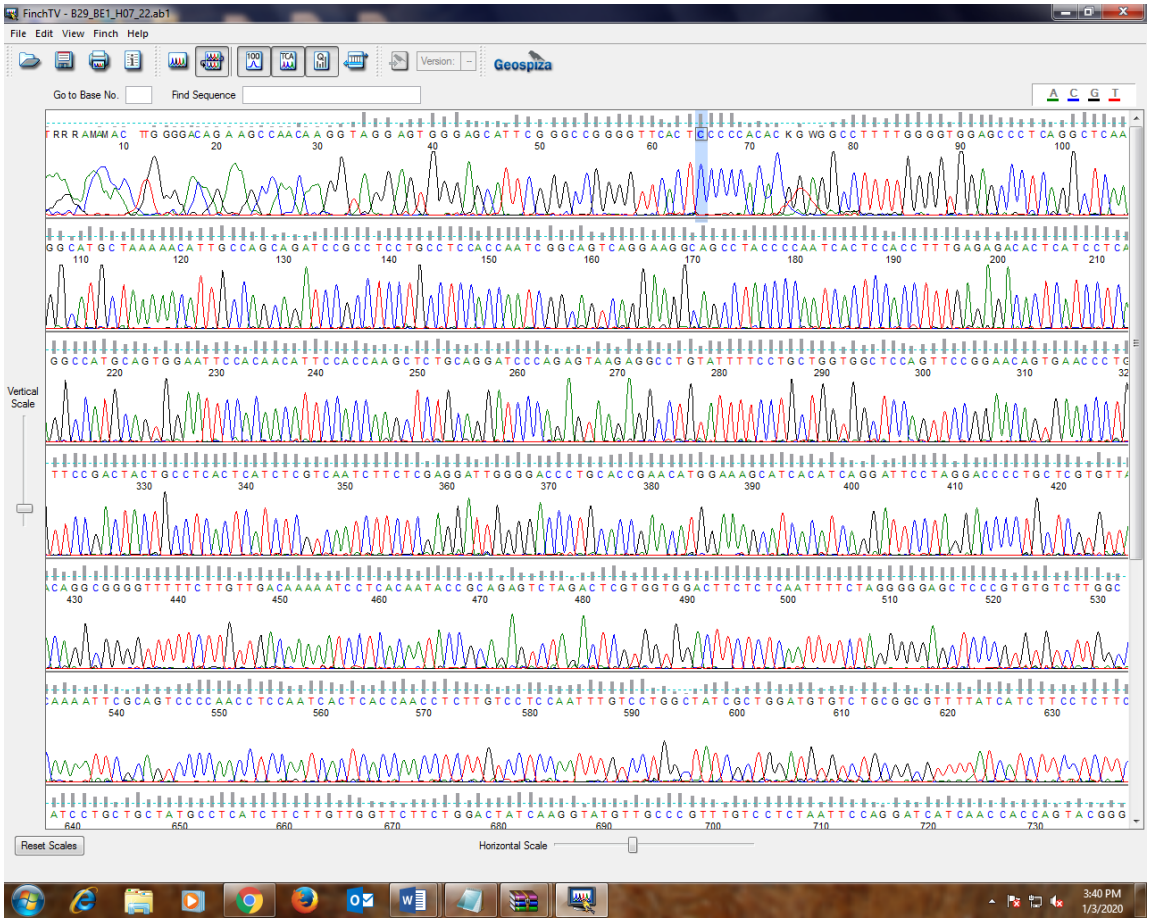


Sample No. 22

CTGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGAGTTCAC

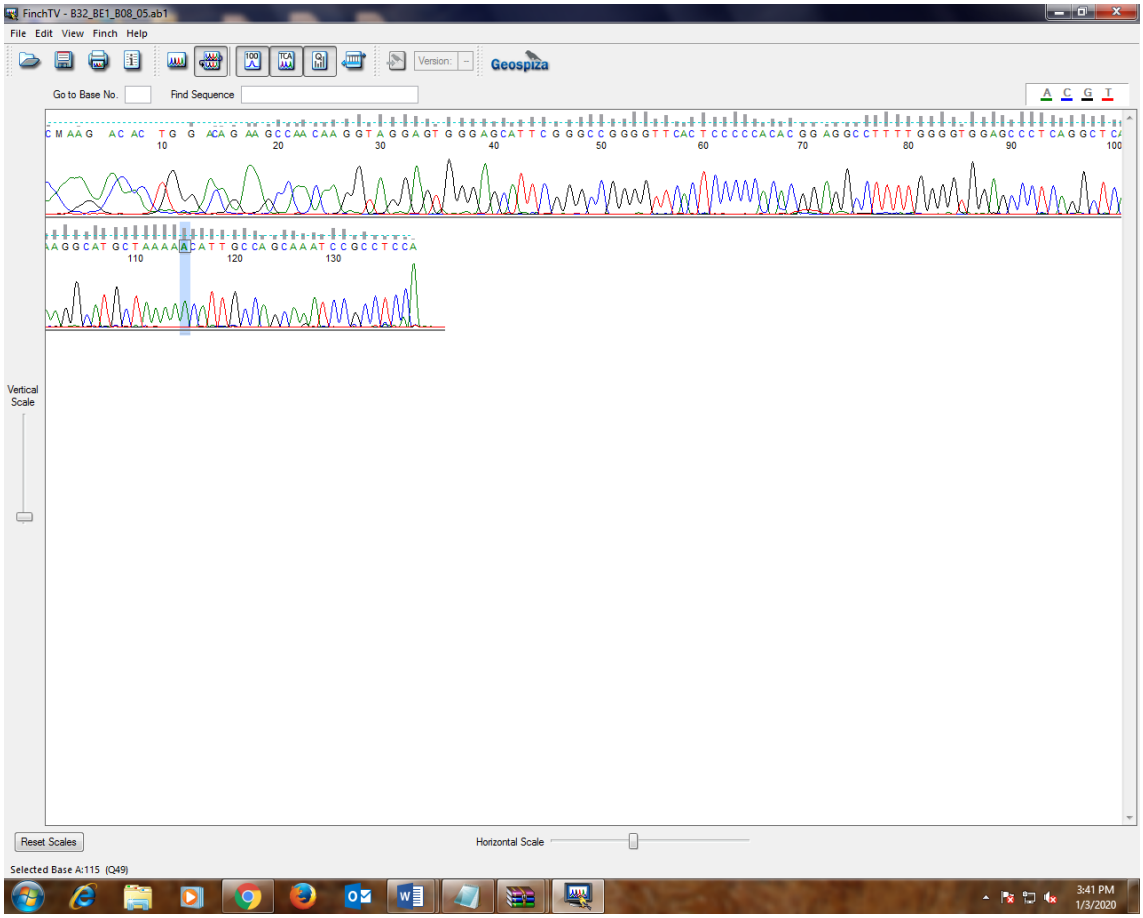
TCCCCCACACGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCT

AAAAACATTGCCAGCAAATCCGCCTCCA



Sample No. 29

GGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGAGGCCTTTTGGGGTG
GAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAGATCCGCCTCC
TGCCTCCACCAATCGGCAGTCAGGAAGGCAGCCTACCCCAATCACTCCACCT
TTGAGAGACACTCATCCTCAGGCCATGCAGTGGAATTCCACAACATTCCACC
AAGCTCTGCAGGATCCCAGAGTAAGAGGCCTGTATTTTCCTGCTGGTGGCTC
CAGTTCCGGAACAGTGAACCCTGTTCCGACTACTGCCTCACTCATCTCGTCA
ATCTTCTCGAGGATTGGGGACCCTGCACCGAACATGGAAAGCATCACATCA
GGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAA
AAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTT
TCTAGGGGGAGCTCCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCC
AATCACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGCTATCGCTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTT
GTTGGTTCTTCTGGACTATCA



Sample No. 32

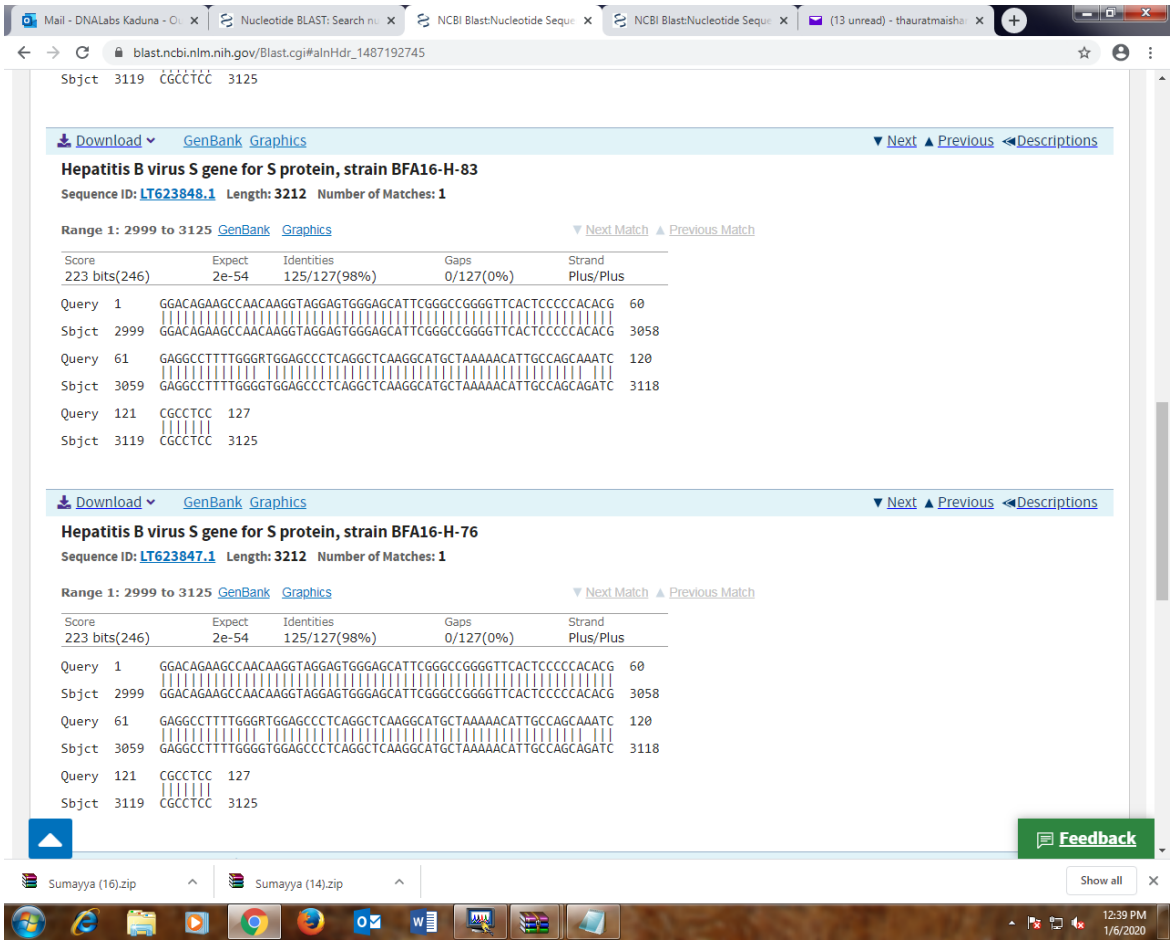
ACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCC

CCACACGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAA

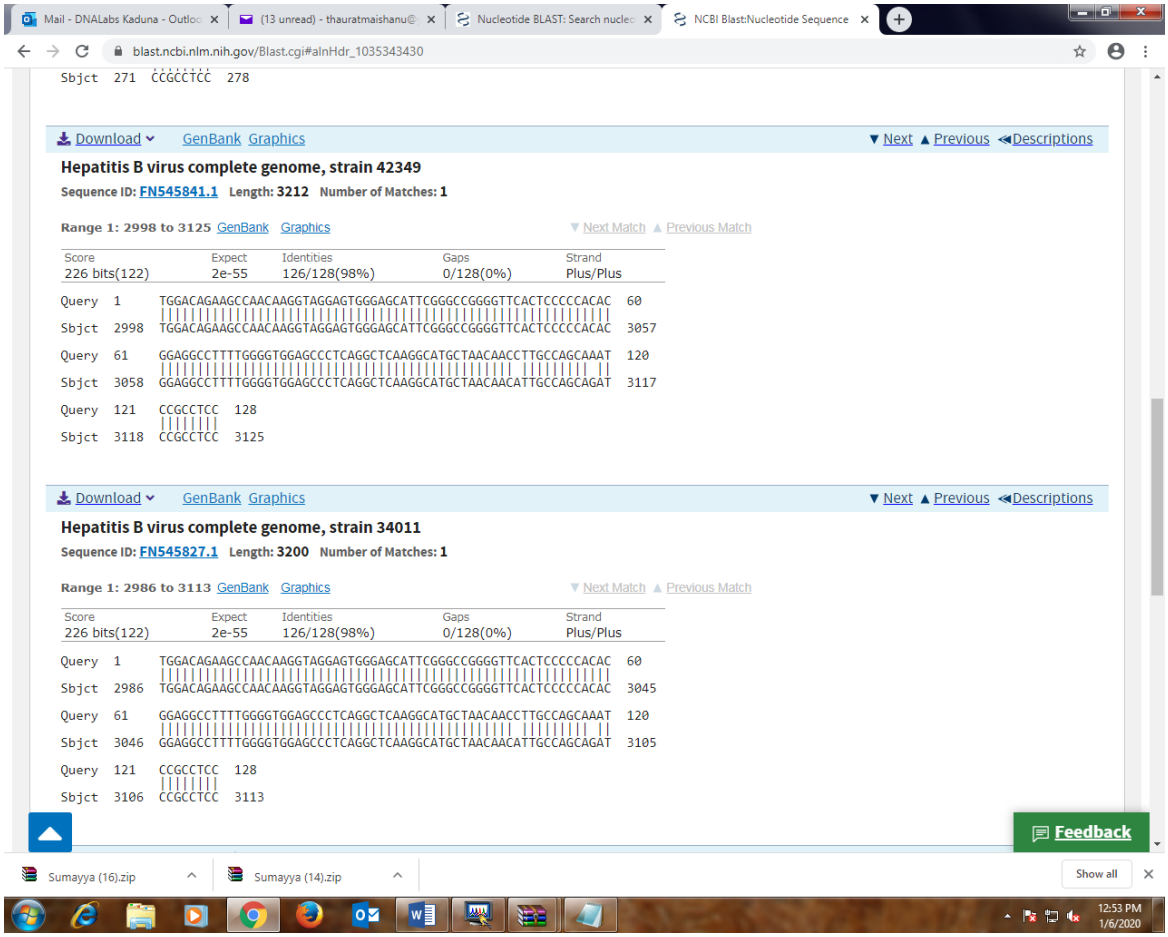
ACATTGCCAGCAAATCCGCCTCCA

Appendix F

Pair-wise Alignment of Five (5) Positive Samples from GenBank identifier



Sample No. 17


Mail - DNALabs Kaduna - Outlo
(13 unread) - thauratmaishanu@
Nucleotide BLAST: Search nucle
NCBI Blast:Nucleotide Sequence
blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1035343430
Sbjct 271 CCGCCTCC 278
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus complete genome, strain 42349
Sequence ID: FN545841.1 Length: 3212 Number of Matches: 1
Range 1: 2998 to 3125 GenBank Graphics
Next Match Previous Match
Score 226 bits(122)
Expect 2e-55
Identities 126/128(98%)
Gaps 0/128(0%)
Strand Plus/Plus
Query 1 TGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACAC 60
Sbjct 2998 TGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACAC 3057
Query 61 GGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAACAACCTTGCCAGCAAAT 120
Sbjct 3058 GGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAACAACATTGCCAGCAGAT 3117
Query 121 CCGCCTCC 128
Sbjct 3118 CCGCCTCC 3125
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus complete genome, strain 34011
Sequence ID: FN545827.1 Length: 3200 Number of Matches: 1
Range 1: 2986 to 3113 GenBank Graphics
Next Match Previous Match
Score 226 bits(122)
Expect 2e-55
Identities 126/128(98%)
Gaps 0/128(0%)
Strand Plus/Plus
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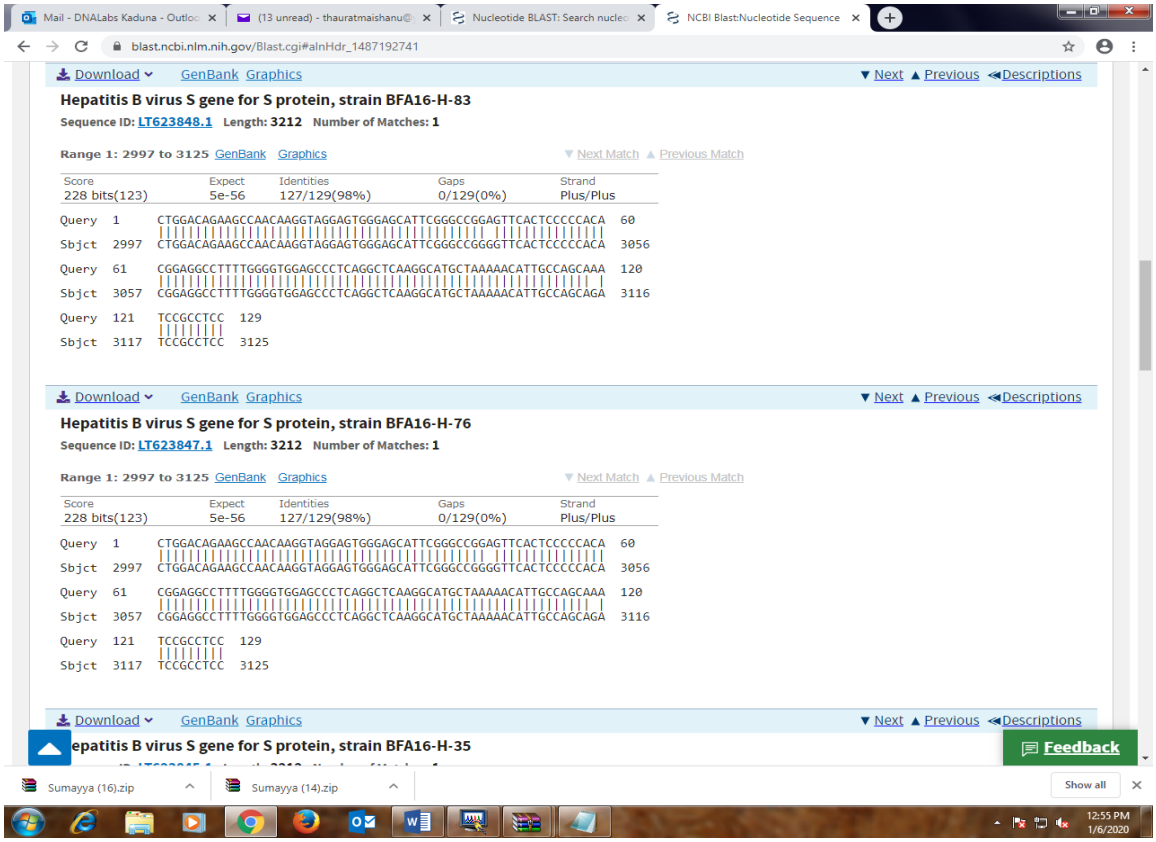
Sbjct 2986 TGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACAC 3045
Query 61 GGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAACAACCTTGCCAGCAAAT 120
Sbjct 3046 GGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAACAACATTGCCAGCAGAT 3105
Query 121 CCGCCTCC 128
Sbjct 3106 CCGCCTCC 3113
Feedback
Sumayya (16).zip
Sumayya (14).zip
Show all
12:53 PM
1/6/2020

Sample No. 19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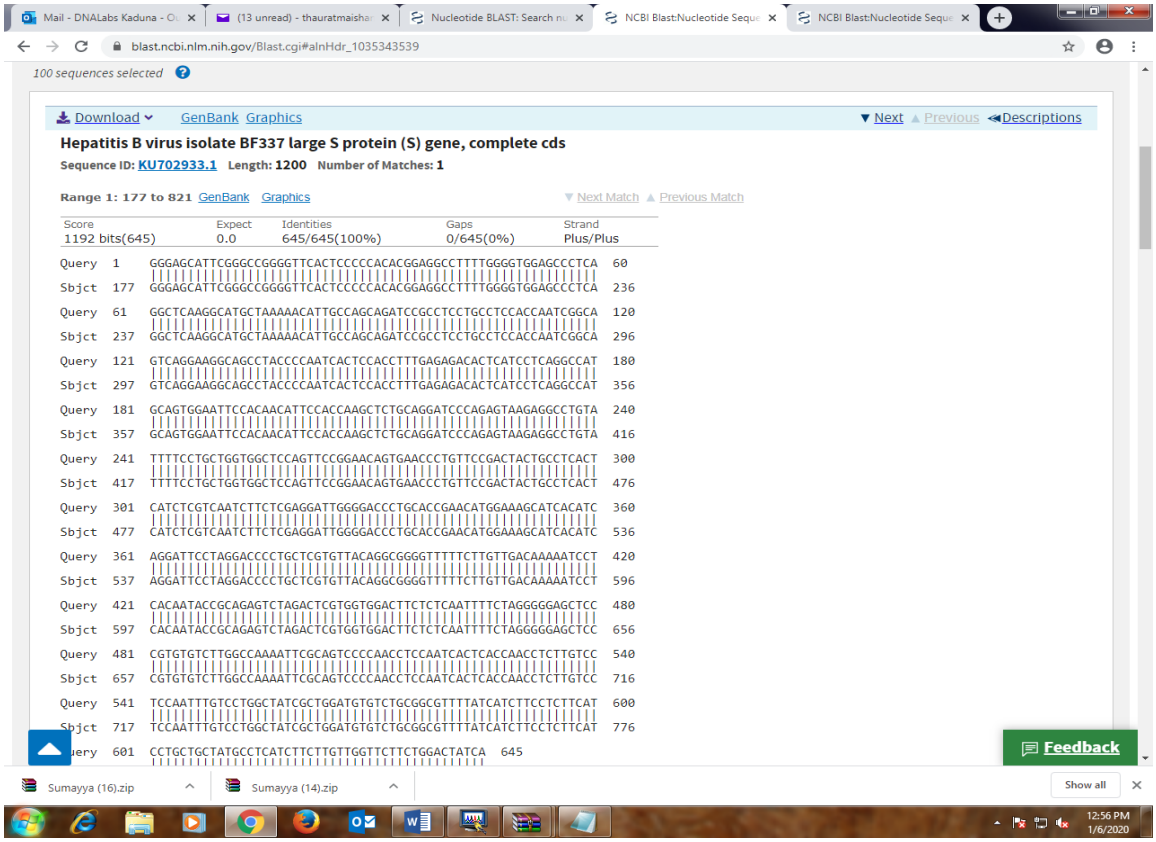
(13 unread) - thauratmaishanu@
Nucleotide BLAST: Search nucle
NCBI Blast:Nucleotide Sequence
blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1487192741
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus S gene for S protein, strain BFA16-H-83
Sequence ID: LT623848.1 Length: 3212 Number of Matches: 1
Range 1: 2997 to 3125 GenBank Graphics
Next Match Previous Match
Score Expect Identities Gaps Strand
228 bits(123) 5e-56 127/129(98%) 0/129(0%) Plus/Plus
Query 1 CTGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGAGTTCACTCCCCCACA 60
Sbjct 2997 CTGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACA 3056
Query 61 CGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAAA 120
Sbjct 3057 CGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAGA 3116
Query 121 TCCGCCTCC 129
Sbjct 3117 TCCGCCTCC 3125
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus S gene for S protein, strain BFA16-H-76
Sequence ID: LT623847.1 Length: 3212 Number of Matches: 1
Range 1: 2997 to 3125 GenBank Graphics
Next Match Previous Match
Score Expect Identities Gaps Strand
228 bits(123) 5e-56 127/129(98%) 0/129(0%) Plus/Plus
Query 1 CTGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGAGTTCACTCCCCCACA 60
Sbjct 2997 CTGGACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACA 3056
Query 61 CGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAAA 120
Sbjct 3057 CGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAGA 3116
Query 121 TCCGCCTCC 129
Sbjct 3117 TCCGCCTCC 3125
Download GenBank Graphics
Next Previous Descriptions
epatitis B virus S gene for S protein, strain BFA16-H-35
Feedback
Sumayya (16).zip
Sumayya (14).zip
Show all
12:55 PM
1/6/2020

Sample No. 22


blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1035343539
100 sequences selected
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus isolate BF337 large S protein (S) gene, complete cds
Sequence ID: KU702933.1 Length: 1200 Number of Matches: 1
Range 1: 177 to 821 GenBank Graphics
Next Match Previous Match
Score 1192 bits(645)
Expect 0.0
Identities 645/645(100%)
Gaps 0/645(0%)
Strand Plus/Plus
Query 1 GGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGAGGCCTTTTGGGGTGGAGCCCTCA 60
Sbjct 177 GGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGAGGCCTTTTGGGGTGGAGCCCTCA 236
Query 61 GGCTCAAGGCATGCTAAAAACATTGCCAGCAGATCCGCCTCCTGCCTCCACCAATCGGCA 120
Sbjct 237 GGCTCAAGGCATGCTAAAAACATTGCCAGCAGATCCGCCTCCTGCCTCCACCAATCGGCA 296
Query 121 GTCAGGAAGGCAGCCTACCCCAATCACTCCACCTTTGAGAGACACTCATCCTCAGGCCAT 180
Sbjct 297 GTCAGGAAGGCAGCCTACCCCAATCACTCCACCTTTGAGAGACACTCATCCTCAGGCCAT 356
Query 181 GCAGTGGAATTCCACAACATTCCACCAAGCTCTGCAGGATCCCAGAGTAAGAGGCCTGTA 240
Sbjct 357 GCAGTGGAATTCCACAACATTCCACCAAGCTCTGCAGGATCCCAGAGTAAGAGGCCTGTA 416
Query 241 TTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTGAACCCTGTTCCGACTACTGCCTCACT 300
Sbjct 417 TTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTGAACCCTGTTCCGACTACTGCCTCACT 476
Query 301 CATCTCGTCAATCTTCTCGAGGATTGGGGACCCTGCACCGAACATGGAAAGCATCACATC 360
Sbjct 477 CATCTCGTCAATCTTCTCGAGGATTGGGGACCCTGCACCGAACATGGAAAGCATCACATC 536
Query 361 AGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAAAATCCT 420
Sbjct 537 AGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAAAATCCT 596
Query 421 CACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAGCTCC 480
Sbjct 597 CACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAGCTCC 656
Query 481 CGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCC 540
Sbjct 657 CGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCC 716
Query 541 TCCAATTTGTCCTGGCTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCAT 600
Sbjct 717 TCCAATTTGTCCTGGCTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCAT 776
Query 601 CCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCA 645
Feedback
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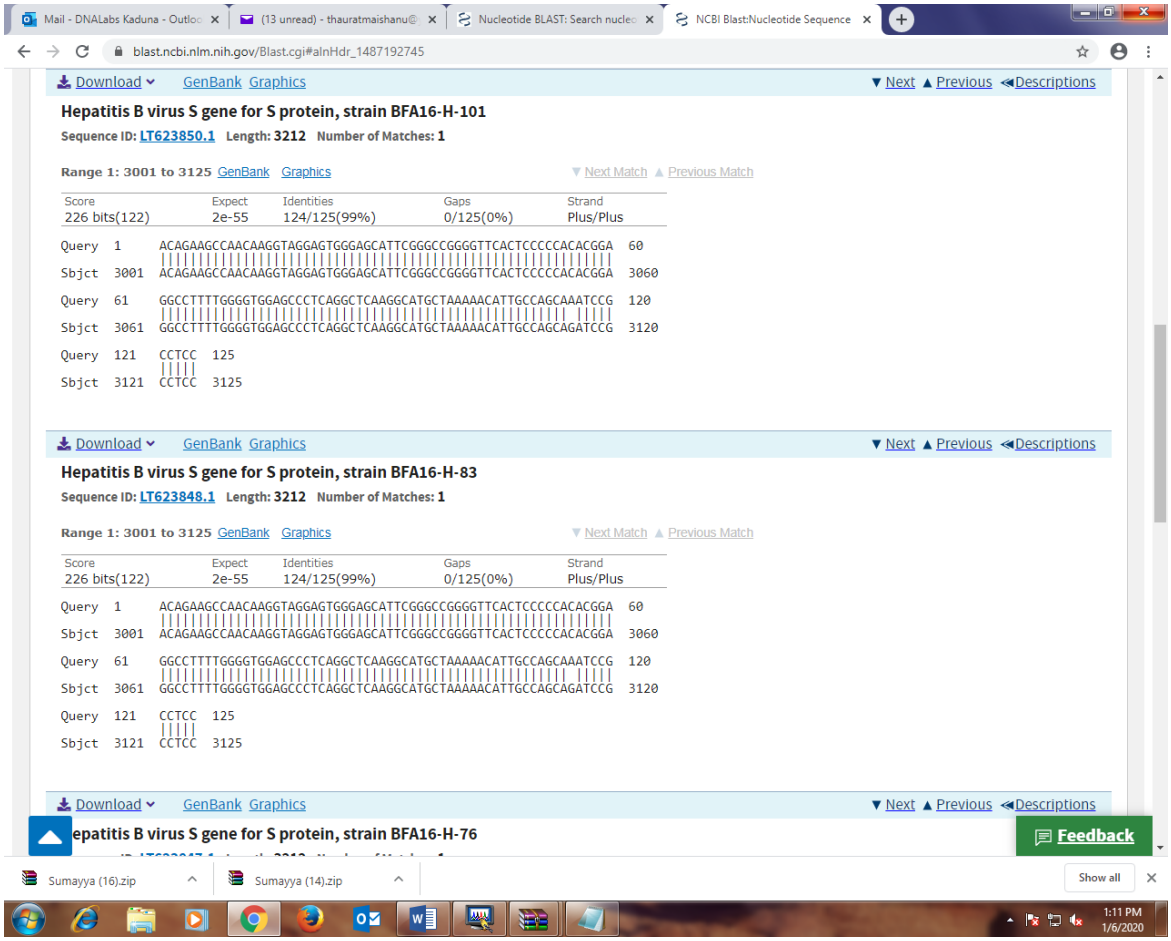
Sumayya (14).zip
Show all
12:56 PM
1/6/2020

Sample No. 29


Mail - DNALabs Kaduna - Outlo
(13 unread) - thauratmaishanu@
Nucleotide BLAST: Search nucle
NCBI Blast:Nucleotide Sequence
blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1487192745
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus S gene for S protein, strain BFA16-H-101
Sequence ID: LT623850.1 Length: 3212 Number of Matches: 1
Range 1: 3001 to 3125 GenBank Graphics
Next Match Previous Match
Score 226 bits(122)
Expect 2e-55
Identities 124/125(99%)
Gaps 0/125(0%)
Strand Plus/Plus
Query 1 ACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGA 60
Sbjct 3001 ACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGA 3060
Query 61 GGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAAATCCG 120
Sbjct 3061 GGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAGATCCG 3120
Query 121 CCTCC 125
Sbjct 3121 CCTCC 3125
Download GenBank Graphics
Next Previous Descriptions
Hepatitis B virus S gene for S protein, strain BFA16-H-83
Sequence ID: LT623848.1 Length: 3212 Number of Matches: 1
Range 1: 3001 to 3125 GenBank Graphics
Next Match Previous Match
Score 226 bits(122)
Expect 2e-55
Identities 124/125(99%)
Gaps 0/125(0%)
Strand Plus/Plus
Query 1 ACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGA 60
Sbjct 3001 ACAGAAGCCAACAAGGTAGGAGTGGGAGCATTCGGGCCGGGGTTCACTCCCCCACACGGA 3060
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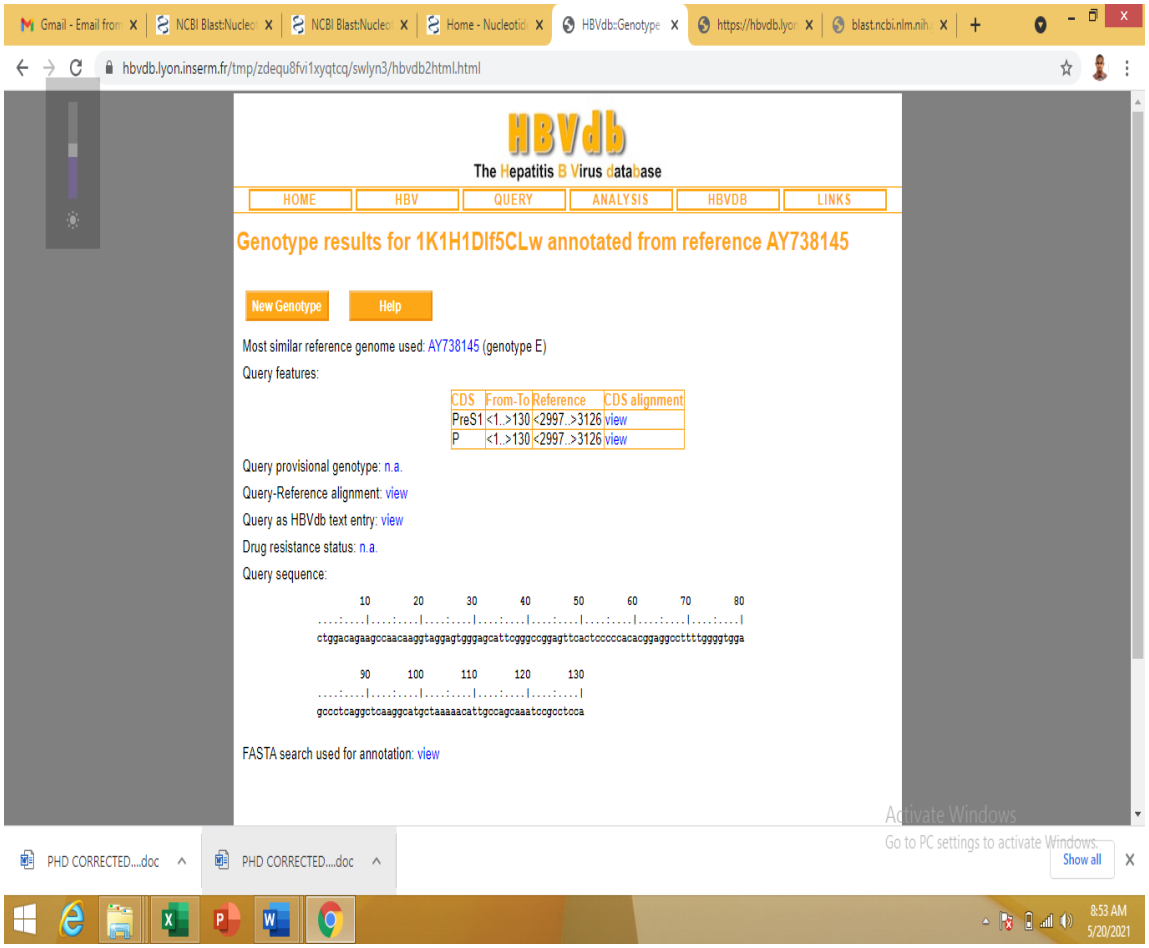
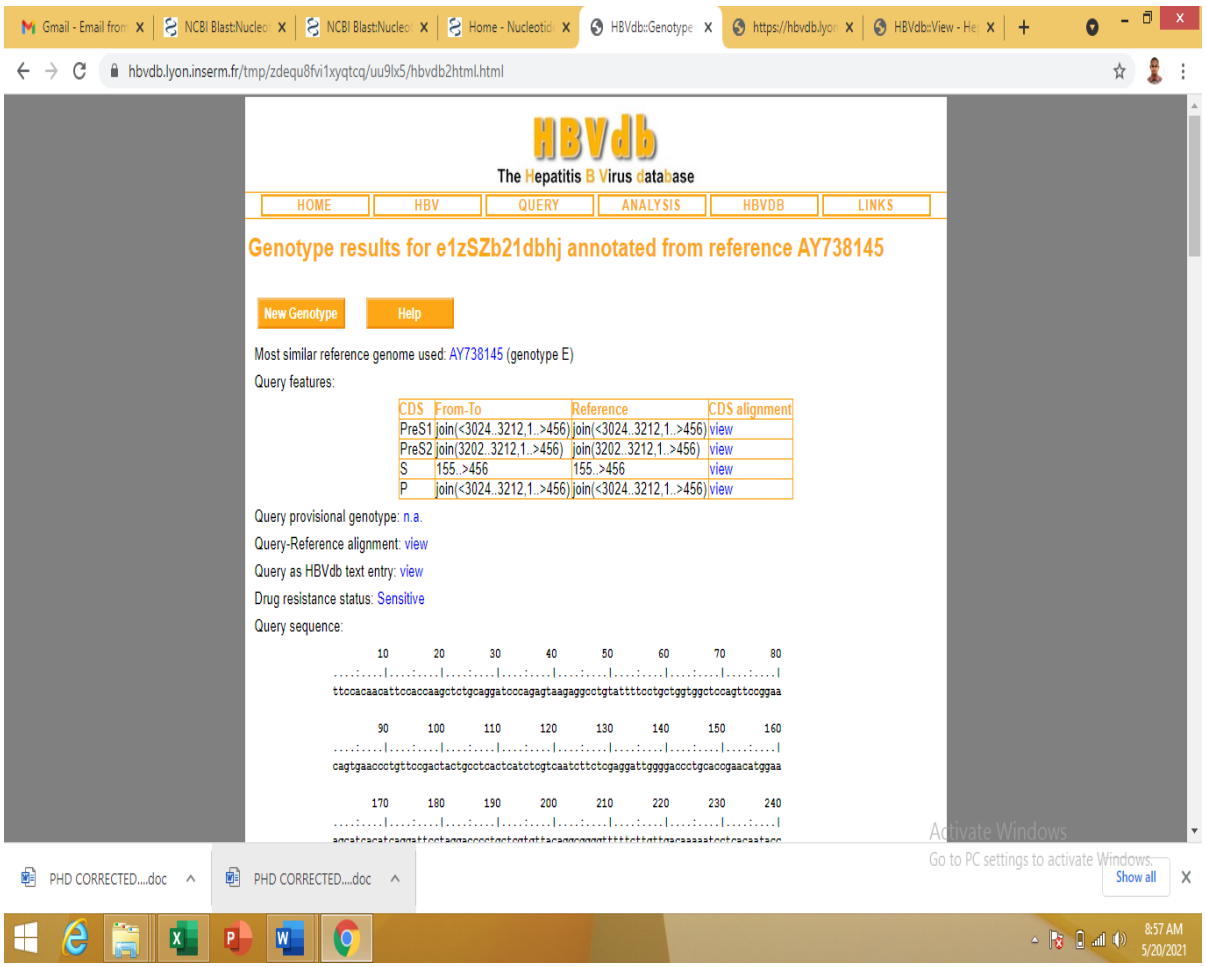
Sbjct 3061 GGCCTTTTGGGGTGGAGCCCTCAGGCTCAAGGCATGCTAAAAACATTGCCAGCAGATCCG 3120
Query 121 CCTCC 125
Sbjct 3121 CCTCC 3125
Download GenBank Graphics
Next Previous Descriptions
epatitis B virus S gene for S protein, strain BFA16-H-76
Feedback
Sumayya (16).zip
Sumayya (14).zip
Show all
1:11 PM
1/6/2020

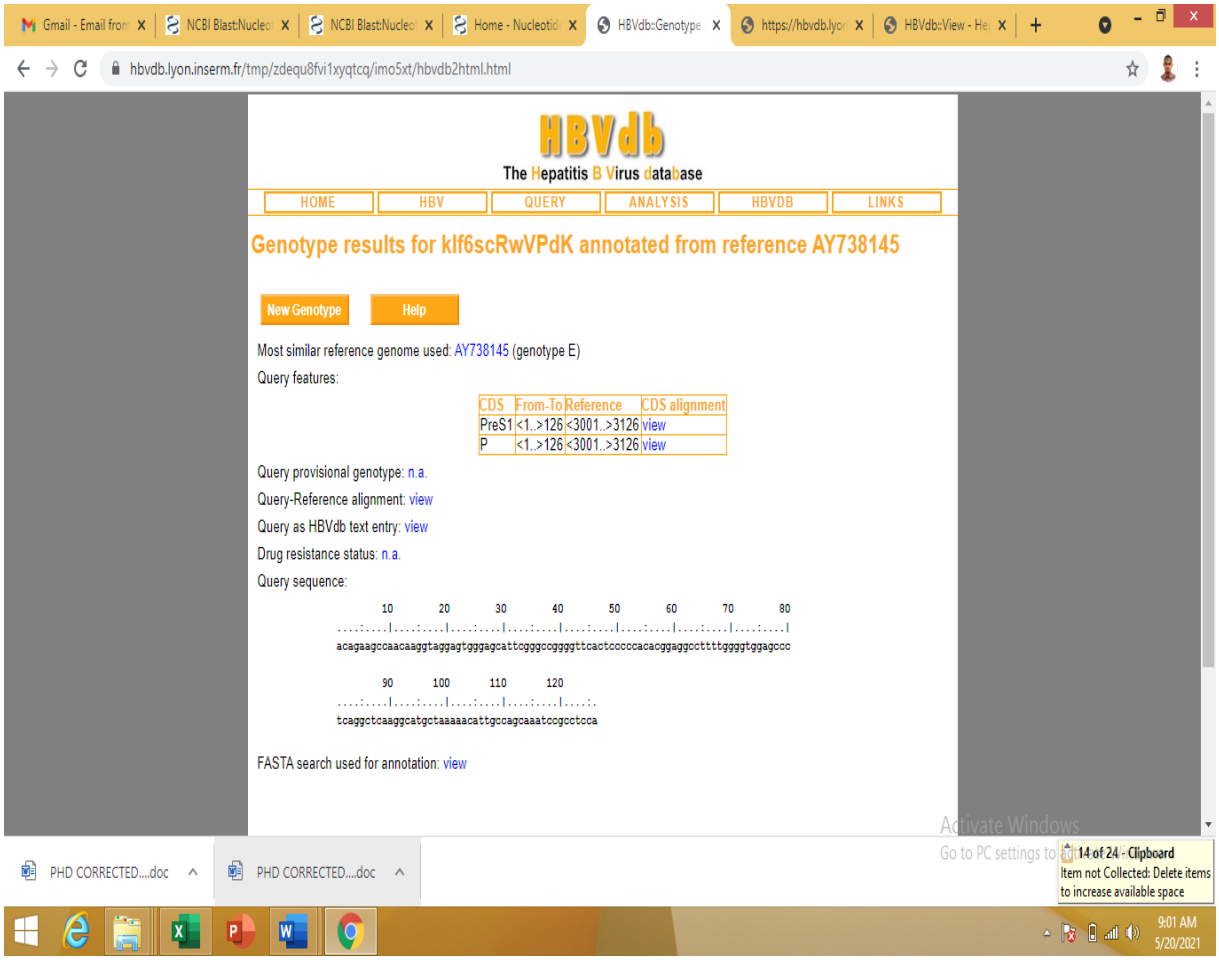
Sample No. 32

Appendix G

Extract of alignment from sequence of five samples and nine reference sequence retrieved from the genbank




Gmail - Email from
NCBI BlastNucleo
NCBI BlastNucleo
Home - Nucleotid
HBVdb::Genotype
https://hbvdb.lyon
HBVdb::View - He
hbvdb.lyon.inserm.fr/tmp/zdequ8fvi1xyqtcq/uu9lx5/hbvdb2html.html
HBVdb
The Hepatitis B Virus database
HOME
HBV
QUERY
ANALYSIS
HBVDB
LINKS
Genotype results for e1zSZb21dbhj annotated from reference AY738145
New Genotype
Help
Most similar reference genome used: AY738145 (genotype E)
Query features:
CDS
From-To
Reference
CDS alignment
PreS1
join(<3024..3212,1..>456)
join(<3024..3212,1..>456)
view
PreS2
join(3202..3212,1..>456)
join(3202..3212,1..>456)
view
S
155..>456
155..>456
view
P
join(<3024..3212,1..>456)
join(<3024..3212,1..>456)
view
Query provisional genotype: n.a.
Query-Reference alignment: view
Query as HBVdb text entry: view
Drug resistance status: Sensitive
Query sequence:
10 20 30 40 50 60 70 80
ttccacaacattccaccaagctctgcaggatcccagagtaagaggcctgtatttcctgctggtggctccagttccggaa
90 100 110 120 130 140 150 160
cagtgaaccctgttccgactactgcctcactcatctcgtcaatcttctcgaggattggggaccctgcacgaacatggaa
170 180 190 200 210 220 230 240
Activate Windows
Go to PC settings to activate Windows.
PHD CORRECTED....doc
PHD CORRECTED....doc
Show all
8:57 AM
5/20/2021


hbvdb.lyon.inserm.fr/tmp/zdequ8fvi1xyqtcq/imo5xt/hbvdb2html.html
HBVdb
The Hepatitis B Virus database
HOME
HBV
QUERY
ANALYSIS
HBVDB
LINKS
Genotype results for klf6scRwVPdK annotated from reference AY738145
New Genotype
Help
Most similar reference genome used: AY738145 (genotype E)
Query features:
CDS From-To Reference CDS alignment
PreS1 <1..>126 <3001..>3126 view
P <1..>126 <3001..>3126 view
Query provisional genotype: n.a.
Query-Reference alignment: view
Query as HBVdb text entry: view
Drug resistance status: n.a.
Query sequence:
10 20 30 40 50 60 70 80
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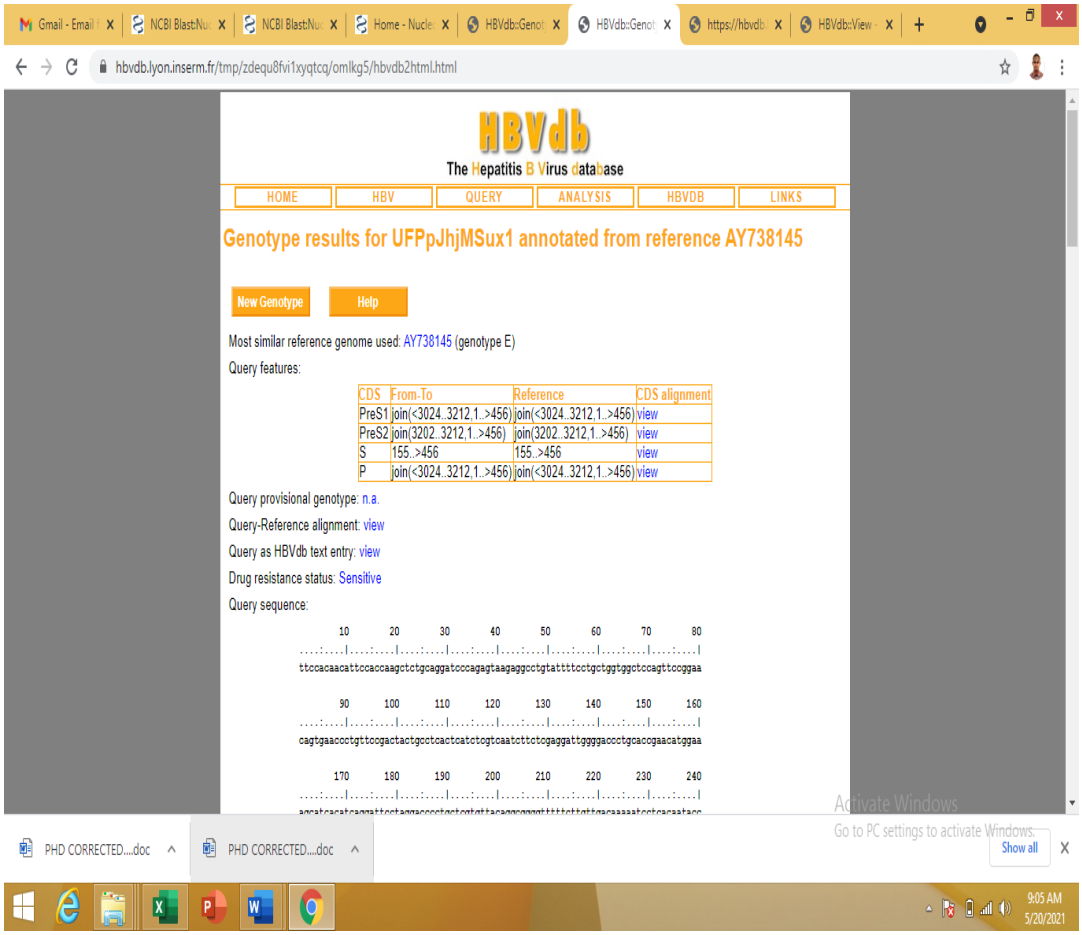
90 100 110 120
tcaggctcaaggcatgctaaaaacattgccagcaaatccgcctcca
FASTA search used for annotation: view
Activate Windows
Go to PC settings to
14 of 24 - Clipboard
Item not Collected: Delete items to increase available space
PHD CORRECTED....doc
PHD CORRECTED....doc
9:01 AM
5/20/2021


HBVdb
The Hepatitis B Virus database
HOME
HBV
QUERY
ANALYSIS
HBVDB
LINKS
Genotype results for UFPpJhjMSux1 annotated from reference AY738145
New Genotype
Help
Most similar reference genome used: AY738145 (genotype E)
Query features:
CDS From-To Reference CDS alignment
PreS1 join(<3024..3212,1..>456) join(<3024..3212,1..>456) view
PreS2 join(3202..3212,1..>456) join(3202..3212,1..>456) view
S 155..>456 155..>456 view
P join(<3024..3212,1..>456) join(<3024..3212,1..>456) view
Query provisional genotype: n.a.
Query-Reference alignment: view
Query as HBVdb text entry: view
Drug resistance status: Sensitive
Query sequence:
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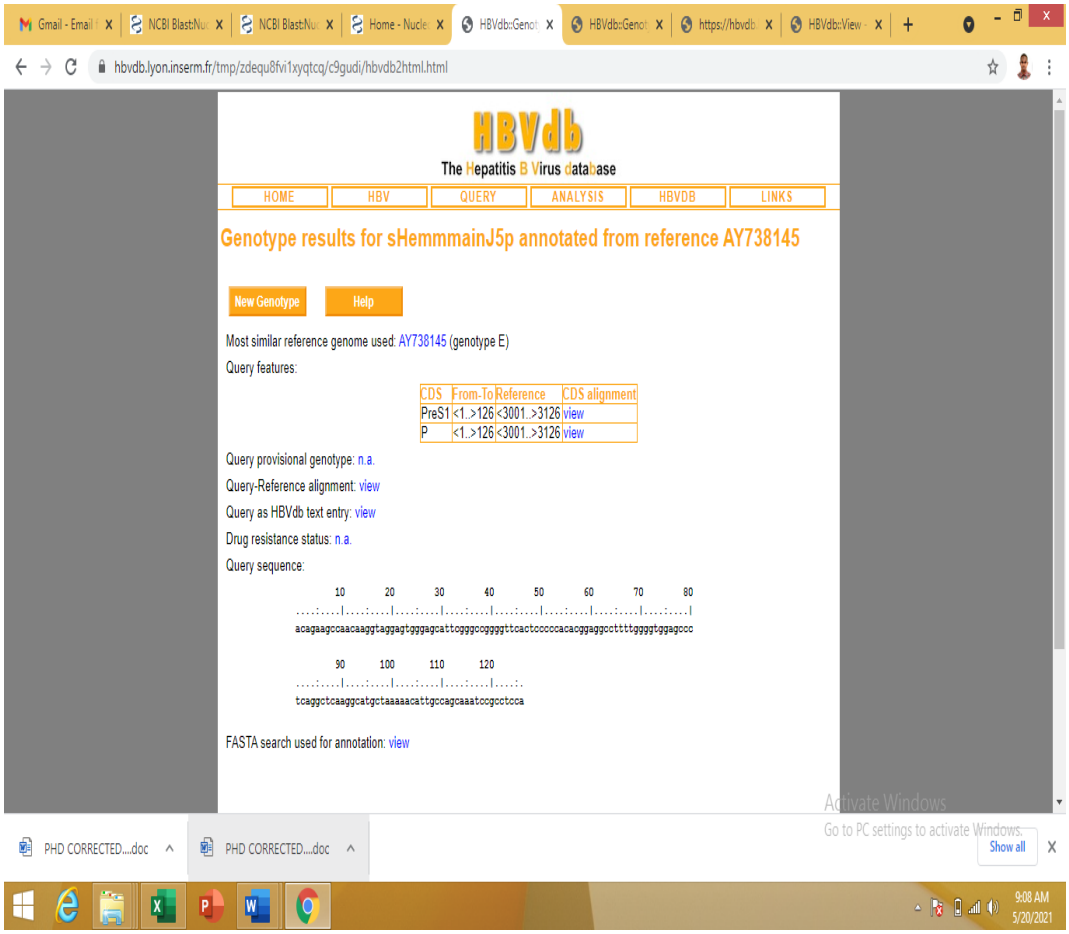
ttccacaacattccaccaagctctgcaggatcccagagtaagaggcctgtattttcctgctggtggctccagttccggaa
90 100 110 120 130 140 150 160
cagtgaacctgttccgactactgcctcactcatctgtcaatcttctcgaggattggggacctgcacgaacatggaa
170 180 190 200 210 220 230 240
Activate Windows
Go to PC settings to activate Windows.
PHD CORRECTED....doc
PHD CORRECTED....doc
Show all
9:05 AM
5/20/2021


Gmail - Email
NCBI BlastNuc
NCBI BlastNuc
Home - Nucle
HBVdb::Genot
HBVdb::Genot
https://hbvdb
HBVdb::View
hbvdb.lyon.inserm.fr/tmp/zdequ8fvi1xyqtcq/c9gudi/hbvdb2html.html
HBVdb
The Hepatitis B Virus database
HOME
HBV
QUERY
ANALYSIS
HBVDB
LINKS
Genotype results for sHemmmainJ5p annotated from reference AY738145
New Genotype
Help
Most similar reference genome used: AY738145 (genotype E)
Query features:
CDS From-To Reference CDS alignment
PreS1 <1..>126 <3001..>3126 view
P <1..>126 <3001..>3126 view
Query provisional genotype: n.a.
Query-Reference alignment: view
Query as HBVdb text entry: view
Drug resistance status: n.a.
Query sequence:
10 20 30 40 50 60 70 80
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90 100 110 120
tcaggctcaaggcatgctaaaaacattgccagcaaatccgcctcca
FASTA search used for annotation: view
Activate Windows
Go to PC settings to activate Windows.
PHD CORRECTED....doc
PHD CORRECTED....doc
Show all
9:08 AM
5/20/2021

APPENDIX H

Linear Regression analysis

Case Processing Summary			
Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	500	99.8
	Missing Cases	1	.2
	Total	501	100.0
Unselected Cases		0	.0
Total		501	100.0
a. If weight is in effect, see classification table for the total number of cases.			

Dependent Variable Encoding

Original Value	Internal Value
Negetive	0
Positive	1

Categorical Variables Codings

		Frequency	Parameter coding (1)	(2)	(3)
Age	18-30	121	1.000	.000	.000
	31-40	136	.000	1.000	.000
	41-50	202	.000	.000	1.000
	51 and above	41	.000	.000	.000
Marital status	Married	455	1.000		
	Single	45	.000		
Occupation	Civil servant	250	1.000		
	Others	250	.000		
H.O. HBV vaccinination	Yes	34	1.000		
	No	466	.000		
Share B&U	Yes	397	1.000		
	No	103	.000		
Blood transfusion	Yes	169	1.000		
	No	331	.000		
	No	351	.000		
Gender	Male	250	1.000		
	Female	250	.000		

Block 0: Beginning Block

Classification Table[a,b]

		Observed	Predicted		
			HBV status		Percentage Correct
			Negetive	Positive	
Step 0	HBV status	Negetive	435	0	100.0
		Positive	65	0	.0
	Overall Percentage				87.0

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-1.901	.133	204.352	1	.000	.149

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Gender(1)	.159	1	.690
		MaritalS(1)	.005	1	.944
		Occupatn(1)	.159	1	.690
		Family(1)	.015	1	.903
		Vaccine(1)	.697	1	.404

	HBV history(1)	.109	1	.741
	Relation(1)	.012	1	.914
	Blood Trans(1)	1.284	1	.257
	Sharing(1)	4.414	1	.036
	Age	.891	3	.828
	Age(1)	.497	1	.481
	Age(2)	.641	1	.423
	Age(3)	.040	1	.841
Overall Statistics		8.312	12	.760

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	8.876	12	.714
	Block	8.876	12	.714
	Model	8.876	12	.714

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	377.511[a]	.018	.033

a. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	13.166	8	.106

Contingency Table for Hosmer and Lemeshow Test

		HBV status = Negetive		HBV status = Positive		Total
		Observed	Expected	Observed	Expected	
Step 1	1	54	52.826	2	3.174	56
	2	46	46.832	5	4.168	51
	3	52	48.398	2	5.602	54
	4	40	44.009	10	5.991	50
	5	43	43.639	7	6.361	50
	6	43	42.276	6	6.724	49
	7	48	46.818	7	8.182	55
	8	33	38.623	13	7.377	46
	9	43	40.959	7	9.041	50
	10	33	30.621	6	8.379	39

Classification Table[a]

	Observed		Predicted		
			HBV status		Percentage Correct
			Negetive	Positive	
Step 1	HBV status	Negetive	435	0	100.0
		Positive	65	0	.0
	Overall Percentage				87.0

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
	Gender(1)	-.056	.382	.021	1	.884	.946	.447	1.999
	MaritalS(1)	.044	.492	.008	1	.928	1.045	.399	2.741
	Occupatn(1)	-.148	.347	.181	1	.670	.863	.437	1.703
Step 1[a]	Vaccine(1)	.317	.522	.369	1	.543	1.373	.494	3.819
	HBV history(1)	-.245	.639	.147	1	.702	.783	.224	2.738
	Relation(1)	.236	.619	.145	1	.704	1.266	.376	4.259
	Blood Trans(1)	.377	.294	1.639	1	.201	1.457	.819	2.594
	Sharing(1)	.909	.421	4.663	1	.031	2.482	1.088	5.665
	Age			1.118	3	.773			
	Age(1)	.068	.648	.011	1	.916	1.071	.300	3.815
	Age(2)	-.296	.615	.232	1	.630	.744	.223	2.482
	Age(3)	.025	.551	.002	1	.964	1.025	.348	3.018
	Constant	-2.741	.879	9.716	1	.002	.065		

Variable(s) entered on step 1: Gender, MaritalS, Occupatn, Family, Vaccine, HBVhistory, Relation, BloodTrans, Sharing razor, Age.

Correlation Matrix

		Constant	Gender(1)	MaritalS(1)	Occupatn(1)	Vaccine(1)	HBV history(1)	Relation(1)	BloodTrans(1)	Sharing(1)	Age	
Step 1	Constant	1.000	.065	-.522	.083	-.116	-.025	-.009		-.212	-.574	-.348
	Gender(1)	.065	1.000	.054	-.453	.254	-.150	.054		.034	-.056	-.296
	MaritalS(1)	-.522	.054	1.000	-.116	.006	-.038	.001		-.011	-.002	-.178
	Occupatn(1)	.083	-.453	-.116	1.000	-.140	.108	-.039		-.150	.010	-.179
	Family(1)	-.088	-.149	-.095	-.013	.148	.236	-.133		-.188	.062	-.147
	Vaccine(1)	-.116	.254	.006	-.140	1.000	-.040	-.046		-.143	-.047	.071
	HBVhistory(1)	-.025	-.150	-.038	.108	-.040	1.000	-.861		-.013	.036	-.089
	Relation(1)	-.009	.054	.001	-.039	-.046	-.861	1.000		.082	-.011	.002
	BloodTrans(1)	-.212	.034	-.011	-.150	-.143	-.013	.082		1.000	.063	.111
	Sharing(1)	-.574	-.056	-.002	.010	-.047	.036	-.011		.063	1.000	-.030
	Age	-.348	-.296	-.178	-.179	.071	-.089	.002		.111	-.030	1.000

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV prevalence in Suleja (%)	100	1.9700	.17145	1.00	2.00

Chi-Square Test

Frequencies

HBV prevalence in Suleja (%)

	Observed N	Expected N	Residual
Positive	3	50.0	-47.0
Negetive	97	50.0	47.0
Total	100		

Test Statistics

	HBV prevalence in Suleja (%)
Chi-Square	88.360[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV prevalence in Minna (%)	100	1.8000	.40202	1.00	2.00

Chi-Square Test

Frequencies

HBV prevalence in Minna (%)

	Observed N	Expected N	Residual
Positive	20	50.0	-30.0
Negetive	80	50.0	30.0
Total	100		

Test Statistics

	HBV prevalence in Minna (%)
Chi-Square	36.000[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV prevalence in Lapai (%)	100	1.8600	.34874	1.00	2.00

Chi-Square Test

Frequencies

HBV prevalence in Lapai (%)

	Observed N	Expected N	Residual
Positive	14	50.0	-36.0
Negetive	86	50.0	36.0
Total	100		

Test Statistics

	HBV prevalence in Lapai (%)
Chi-Square	51.840[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV prevalence in Kontagora (%)	100	1.8800	.32660	1.00	2.00

Chi-Square Test

Frequencies

HBV prevalence in Kontagora (%)

	Observed N	Expected N	Residual
Positive	12	50.0	-38.0
Negetive	88	50.0	38.0
Total	100		

Test Statistics

	HBV prevalence in Kontagora (%)
Chi-Square	57.760[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV prevalence in Bida (%)	100	1.8400	.36845	1.00	2.00

Chi-Square Test

Frequencies

HBV prevalence in Bida (%)

	Observed N	Expected N	Residual
Positive	16	50.0	-34.0
Negetive	84	50.0	34.0
Total	100		

Test Statistics

	HBV prevalence in Bida (%)
Chi-Square	46.240[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV frequency/Suleja (%)	6	2.0000	.00000	2.00	2.00

Chi-Square Test

Frequencies

HBV frequency/Suleja (%)

	Observed N	Expected N	Residual
Female	6	6.0	.0
Total	6[a]		

a. This variable is constant. Chi-Square Test cannot be performed.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV frequency/Minna (%)	40	1.4000	.49614	1.00	2.00

Chi-Square Test

Frequencies

HBV frequency/Minna (%)

	Observed N	Expected N	Residual
Male	24	20.0	4.0
Female	16	20.0	-4.0
Total	40		

Test Statistics

	HBV frequency/Minna (%)
Chi-Square	1.600[a]
Df	1
Asymp. Sig.	.206
Exact Sig.	.268
Point Probability	.114

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 20.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV frequency/Lapai (%)	28	1.3571	.48795	1.00	2.00

Chi-Square Test

Frequencies

HBV frequency/Lapai (%)

	Observed N	Expected N	Residual
Male	18	14.0	4.0
Female	10	14.0	-4.0
Total	28		

Test Statistics

	HBV frequency/Lapai (%)
Chi-Square	2.286[a]
Df	1
Asymp. Sig.	.131
Exact Sig.	.185
Point Probability	.098

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 14.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV frequency/Kontagora (%)	24	1.7500	.44233	1.00	2.00

Chi-Square Test

Frequencies

HBV frequency/Kontagora (%)

	Observed N	Expected N	Residual
Male	6	12.0	-6.0
Female	18	12.0	6.0
Total	24		

Test Statistics

	HBV frequency/Kontagora (%)
Chi-Square	6.000[a]
Df	1
Asymp. Sig.	.014
Exact Sig.	.023
Point Probability	.016

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 12.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV frequency/Bida (%)	32	1.5625	.50402	1.00	2.00

Chi-Square Test

Frequencies

HBV frequency/Bida (%)

	Observed N	Expected N	Residual
Male	14	16.0	-2.0
Female	18	16.0	2.0
Total	32		

Test Statistics

	HBV frequency/Bida (%)
Chi-Square	.500[a]
Df	1
Asymp. Sig.	.480
Exact Sig.	.597
Point Probability	.220

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 16.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	17	1.8235	.39295	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Suleja	3	8.5	-5.5
Lapai	14	8.5	5.5
Total	17		

Test Statistics

	HBV Prevalence (%)
Chi-Square	7.118[a]
Df	1
Asymp. Sig.	.008
Exact Sig.	.013
Point Probability	.010

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 8.5.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	23	1.8696	.34435	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Suleja	3	11.5	-8.5
Minna	20	11.5	8.5
Total	23		

Test Statistics

	HBV Prevalence (%)
Chi-Square	12.565[a]
Df	1
Asymp. Sig.	.000
Exact Sig.	.000
Point Probability	.000

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 11.5.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	15	1.8000	.41404	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Suleja	3	7.5	-4.5
Kontagora	12	7.5	4.5
Total	15		

Test Statistics

	HBV Prevalence (%)
Chi-Square	5.400[a]
Df	1
Asymp. Sig.	.020
Exact Sig.	.035
Point Probability	.028

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 7.5.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	19	1.8421	.37463	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Suleja	3	9.5	-6.5
Bida	16	9.5	6.5
Total	19		

Test Statistics

	HBV Prevalence (%)
Chi-Square	8.895[a]
Df	1
Asymp. Sig.	.003
Exact Sig.	.004
Point Probability	.004

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 9.5.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	36	1.4444	.50395	1.00	2.00

Chi-Square Test

	HBV Prevalence (%)
Chi-Square	.444[a]
Df	1
Asymp. Sig.	.505
Exact Sig.	.618
Point Probability	.213

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 18.0.

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Minna	20	18.0	2.0
Bida	16	18.0	-2.0
Total	36		

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	32	1.3750	.49187	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Minna	20	16.0	4.0
Kontagora	12	16.0	-4.0
Total	32		

Test Statistics

	HBV Prevalence (%)
Chi-Square	2.000[a]
Df	1
Asymp. Sig.	.157
Exact Sig.	.215
Point Probability	.105

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 16.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	34	1.4118	.49955	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Minna	20	17.0	3.0
Lapai	14	17.0	-3.0
Total	34		

Test Statistics

	HBV Prevalence (%)
Chi-Square	1.059[a]
Df	1
Asymp. Sig.	.303
Exact Sig.	.392
Point Probability	.162

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 17.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	26	1.5385	.50839	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Kontagora	12	13.0	-1.0
Lapai	14	13.0	1.0
Total	26		

Test Statistics

	HBV Prevalence (%)
Chi-Square	.154[a]
Df	1
Asymp. Sig.	.695
Exact Sig.	.845
Point Probability	.288

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 13.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	28	1.5714	.50395	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Kontagora	12	14.0	-2.0
Bida	16	14.0	2.0
Total	28		

Test Statistics

	HBV Prevalence (%)
Chi-Square	.571[a]
Df	1
Asymp. Sig.	.450
Exact Sig.	.572
Point Probability	.227

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 14.0.

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
HBV Prevalence (%)	30	1.5333	.50742	1.00	2.00

Chi-Square Test

Frequencies

HBV Prevalence (%)

	Observed N	Expected N	Residual
Lapai	14	15.0	-1.0
Bida	16	15.0	1.0
Total	30		

Test Statistics

	HBV Prevalence (%)
Chi-Square	.133[a]
Df	1
Asymp. Sig.	.715
Exact Sig.	.856
Point Probability	.271