MOLECULAR EPIDEMIOLOGY OF HEPATITIS B AND HEPATITIS D IN KELANTAN, MALAYSIA

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MOLECULAR EPIDEMIOLOGY OF HEPATITIS B AND HEPATITIS D IN KELANTAN, MALAYSIA

by

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cccDNA	Covalently Closed Circular DNA (HBV replication intermediate)
pgRNA	Pregenomic RNA (HBV replication intermediate)
RT	Reverse Transcriptase
HBx	Hepatitis B X Protein
HBs	Hepatitis B Surface Protein
Pol	Polymerase Protein
RC-DNA	Relaxed Circular DNA
STING	Stimulator of Interferon Genes
HBV-HDV	HBV and HDV Coinfection
HBV-HIV	HBV and HIV Coinfection
HBVseq	HBV Sequence Database
rtM204V/I	Mutation in the reverse transcriptase region, associated with lamivudine resistance.
rtL180M	Mutation commonly seen with rtM204V/I, enhances drug resistance.
rtA181T/V	Mutations associated with adefovir and tenofovir resistance.
rtN236T	Mutation linked to adefovir resistance.
rtS202I/G	Mutations contributing to entecavir resistance.
rtT184G/A	Associated with entecavir resistance.
tV207I	Mutation that may emerge during lamivudine or telbivudine treatment.
rtQ215S	Mutation associated with reduced susceptibility to adefovir.
sG145R	Mutation in the “a” determinant of HBsAg, causing vaccine escape and diagnostic failure.
sP120T	Mutation affecting HBV surface antigen detection and immune evasion.

G1896A	Pre-core mutation, resulting in the stop codon that blocks HBeAg production, linked to HBeAg-negative chronic hepatitis B.
A1762T	Basal core promoter (BCP) mutations, leading to reduced HBeAg production.
K130M	Mutations in the X gene linked to HBV-related liver disease, including hepatocellular carcinoma (HCC).
A138T	Mutation affecting the function of the HBx protein, involved in oncogenesis.
V131I	Mutation potentially linked to advanced liver disease.
VEM	Vaccine escape mutation
IEM	Immune escape mutation
DFM	Drug function mutation
ARM	Antiviral resistance mutation

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ABSTRAK

Beban global virus hepatitis B (HBV) dan virus hepatitis D (HDV) masih mempunyai kepentingan epidemiologi. Epidemiologi molekul HBV dan HDV di Malaysia masih belum didokumenkan dengan baik, dan tiada laporan mengenai kepelbagaian genomik HBV dan HDV dalam kohort HBV kronik di Kelantan walaupun terdapat insiden jangkitan HBV yang tinggi di rantau ini. HBV kronik di Kelantan dan mutasi ubat HBV yang berkaitan di Kelantan. Sampel darah telah dikumpul daripada 226 pesakit HBV kronik di Kelantan dan disaring untuk antibodi HBV dan HDV menggunakan teknik ujian imunosorben berkaitan enzim (ELISA). DNA/RNA telah diekstrak daripada sampel, dan RNA HDV ditukar kepada cDNA menggunakan kit sintesis cDNA. DNA/cDNA subjek telah dikuatkan menggunakan kaedah PCR bersarang. Semua amplikon telah dielektroforesis dan dijujukan. Jujukan telah ditaip menggunakan pokok Phylogenetic dan alat subtaip (alat genotaip NCBI) ke dalam genotip, subjenis dan serotaip masing-masing dengan sewajarnya. Mutasi berkaitan ubat HBV telah dijalankan menggunakan alat bioinformatik (Gene2Pheno dan JpHMM GOBICS). Tiga genotip HBV (genotip HBV B, C, dan D) dan dua genotip HBV rekombinan (genotip HBV B/A dan genotip HBV B/C) telah ditemui dalam edaran di Kelantan. Genotip HBV B mempunyai prevalens tertinggi (93.0%), manakala genotip HBV D mempunyai prevalens paling rendah (1.0%). HBV sub-genotip B3 (31.0%) mempunyai kejadian tertinggi di Kelantan. Dinamik evolusi HBV

mendedahkan serotiip HBV yang berbeza dalam edaran dalam populasi kajian. Serotaip adw (54.0%) adalah serotaip utama dalam kajian ini. Dua genotip HDV [genotip HDV I (40.0%) dan genotip HDV II (60.0%)] sedang dalam edaran di Kelantan. Pokok masa filogenetik mendedahkan bahawa genotip HBV B bermula sejak kira-kira 400 tahun yang lalu, manakala genotip HBV C dan D agak baru, masing-masing 40 tahun dan 11 tahun. Genotip HDV berada dalam kumpulan genomik kira-kira 40 tahun yang lalu. Kota Bharu, Tumpat dan Kuala Krai merupakan kawasan berisiko geografi untuk memperoleh genotip HBV dan HDV. Umur, Pekerjaan, jantina, status perkahwinan dan penanda biokimia (Jumlah dan bilirubin langsung, AST, dan ALP) didapati menjadi faktor risiko predisposisi dalam memperoleh genotip HBV dan HDV di Kelantan pada $P<0.05$. Terdapat bukti tentang rintangan yang berkaitan dengan dadah di kalangan pesakit naif dan dirawat termasuk dalam kajian ini. Rintangan ubat Lamivudine adalah yang tertinggi (26.6%), manakala rintangan tenofovir adalah yang paling rendah (9.04%). Kelaziman genotip HBV dan kepelbagaian evolusi di Kelantan mempunyai kepentingan epidemiologi, dan pengawasan dan pemantauan genomik harus dilaksanakan. Kehadiran HDV di Kelantan menekankan keperluan untuk pengawasan, pemantauan dan kawalan HDV rutin di Kelantan dan Malaysia.

MOLECULAR EPIDEMIOLOGY OF HEPATITIS B AND HEPATITIS D IN KELANTAN, MALAYSIA

ABSTRACT

The global burden of hepatitis B virus (HBV) and hepatitis D virus (HDV) is still of epidemiological significance. The molecular epidemiology of HBV and HDV in Malaysia has yet to be well documented. This study aims to determine the molecular epidemiology of HBV and HDV among a chronic HBV cohort in Kelantan and the associated HBV drug mutation within Kelantan. Blood samples were collected from 226 chronic HBV patients in Kelantan and screened for HBV and HDV antiantibodies using an enzyme-linked immunosorbent assay (ELISA) technique. DNA/RNA was extracted from the samples, and the HDV RNA was converted to cDNA using a cDNA synthesis kit. The DNA/cDNA of the subjects were amplified using a nested PCR method. All amplicons were electrophoresed and sequenced. The sequences were typed using Phylogenetic tree and subtyping tools into their respective genotypes, subtypes, and serotypes accordingly. Three HBV genotypes (HBV genotypes B, C, and D) and two recombinant HBV genotypes (HBV genotype B/A and HBV genotype B/C) were discovered to be in circulation within Kelantan. HBV genotype B had the highest prevalence (93.0%), while HBV genotype D had the lowest prevalence (1.0%). HBV sub-genotype B3 (31.0%) had the highest occurrence in Kelantan. Evolutionary dynamics of HBV reveal different serotypes of HBV in circulation within the study population. Serotype adw (54.0%) was the predominant serotype in this study. Two HDV genotypes [HDV genotype I (40.0%) and HDV genotype II (60.0%)] were in

circulation in Kelantan. The phylogenetic time tree revealed that HBV genotype B dates back approximately 400 years ago, while HBV genotypes C and D were relatively recent, 40 years and 11 years, respectively. HDV genotypes were in the genomic pool approximately 40 years ago. Kota Bharu, Tumpat, and Kuala Krai were the geographic risk regions for acquiring HBV and HDV genotypes. Age, Occupation, gender, marital status, and biochemical markers (Total and direct bilirubin, AST, and ALP) were discovered to be predisposing risk factors in acquiring HBV and HDV genotypes within Kelantan at $P<0.05$. There was evidence of drug-associated resistance among the naïve and treated patients included in this study. Lamivudine drug resistance was the highest (26.6%), while tenofovir resistance was the lowest (9.04%). The prevalence of HBV genotypes and evolutionary diversity in Kelantan is of epidemiological significance, and genomic surveillance and monitoring should be implemented. The presence of HDV in Kelantan stresses the need for routine HDV surveillance and monitoring in Kelantan and Malaysia.

CHAPTER 1

RESEARCH BACKGROUND

1.1 Background of research

Hepatitis B virus (HBV) infection is still of great concern despite the introduction of hepatitis B vaccination in the late 90s. Chronic HBV infections cause liver-related medical complications, such as hepatocellular carcinoma, cirrhosis, and liver failure (Li et al., 2021; Raihan, 2016). Over 240 million cases of HBV infection have been reported globally, and approximately 14 million of these cases were co-infected with hepatitis D virus (HDV), with a mortality index greater than 10% globally with approximately 600,000 HBV or HDV-related deaths annually (Rizzetto, 2018; Zhang & Urban, 2020).

HDV is a defective RNA virus, the only single genus of the member Delta virus. HDV can only exist in the presence of a hepatitis B surface antigen (Stockdale et al., 2020). HBV is a significant public health concern in Malaysia, posing a considerable burden on the population and healthcare system. Malaysia has been grappling with the challenges of preventing and managing HBV infections for many years (zhi-Lim et al., 2022). This viral infection primarily affects the liver and can lead to severe health consequences, including chronic liver disease, cirrhosis, and liver cancer (Kristina et al., 2021). The prevalence of Hepatitis B in Malaysia remains a matter of concern despite the introduction of vaccination in 1989.

Malaysia's diverse population, with a mix of various ethnic groups and cultural practices, contributes to the complexity of managing Hepatitis B. Certain communities

in Malaysia have a higher prevalence of the infection due to factors such as low awareness, inadequate access to healthcare, and cultural practices that may facilitate viral transmission (Muhamad et al., 2020).

These challenges make it imperative for the Malaysian healthcare system to implement targeted prevention and control measures to mitigate the impact of Hepatitis B (Rajamoorthy et al., 2020).

The high degree of genomic heterogeneity categorizes HBV into ten genotypes (A-J), and an intergroup difference of around 7.5% is observed (Kafeero et al., 2022). All genotypes, except E and G, are classified further into 25 different sub-genotypes, with a difference of around 4% in amino acid variability observed in HBV-B. HBV-C is generally found in Oceania and Eastern Asia, while HBV-E is in both Central and Western Africa (Kafeero et al., 2022). HBV-F and HBV-H are found in Alaska and Latin America only. HBV-D is a pandemic. HBV genotype D, subgenotype D1, dominates in Australia, Europe, Indonesia, North Africa, and Western Asia, whereas HBV-genotype D, subgenotype D2, is seen in Albania, Japan, Malaysia, North-Eastern Europe, Russia, and the United Kingdom (Sunbul, 2014). A recent study in Brazil showed that the dominance of genotype D/D3 is observed due to Italian colonization. A similar study was performed before in the same region, and once again, genotype D/D3 was found to be the most relevant genotype (Lampe et al., 2017).

One of the key factors influencing the clinical outcomes and transmission dynamics of HBV is its genetic diversity, characterized by distinct genotypes. Understanding the genotypes prevalent in Asia is crucial for assessing disease progression, treatment response, and vaccine effectiveness, ultimately informing

strategies for preventing, diagnosing, and managing HBV infection (Pujol et al., 2020). HBV and HDV have the same route of transmission, which includes sexual intercourse, mother-to-child transmission, blood transfusion, and the use of intravenous drugs. Dual infection of HBV and HDV can be acquired concurrently with one another, as in the case of a co-infection, which in many instances could be acute (Robinson et al., 2023). The dual infection often has minimal hepatic damage due to the clearance of the viruses in about 90% of such cases. Superinfection is also a common way of acquiring dual infection; in this case, HDV infection is acquired by individuals already having a Hepatitis B virus infection. Many of these cases (over 60%) often progress into chronic hepatitis with high hepatic damage (Sausen et al., 2022).

In Malaysia, HDV represents an additional challenge in the fight against viral hepatitis, as it exacerbates the severity of liver disease and significantly burdens affected individuals and the healthcare system. Understanding HDV's prevalence, risk factors, and impact in Malaysia is crucial for implementing effective prevention and control measures (Hudu et al., 2018). The transmission dynamics of HDV closely follow those of HBV, primarily occurring through percutaneous exposure to infected blood or sexual contact. Individuals at higher risk for HDV infection in Malaysia include injection drug users, healthcare workers, and those with a history of multiple sexual partners (Hudu et al., 2018; Tan et al., 1989).

The co-infection of HDV with HBV can lead to more severe liver disease, including increased chronic liver inflammation, cirrhosis, and hepatocellular carcinoma (HCC). These complications impose a substantial health and economic

burden on affected individuals and the healthcare system in Malaysia (Wungu et al., 2019). Efforts to address HDV in Malaysia focus on enhancing the prevention and control of HBV, as HDV infection depends on HBV co-infection. Collaborating with healthcare organizations, the Malaysian government has implemented comprehensive strategies to control HBV transmission, including universal vaccination programs, blood screening protocols, and harm reduction initiatives for high-risk populations. These measures aim to reduce HBV's overall burden and minimize the risk of HDV co-infection (Negro, 2014; Wedemeyer et al., 2023).

There are two genotypes of HDV in circulation in Southeast Asia, Genotypes 1 and 2. Genotype 1 is the most prevalent genotype of HDV in Thailand (Posuwan et al., 2016). It is primarily associated with co-infection with HBV genotypes B and C, which are the predominant HBV genotypes in the country. Genotype 1 is characterized by diverse subtypes, indicating the presence of genetic variations within this genotype (Amini-Bavil-Olyaee et al., 2011; Su et al., 2006). Genotype 2 of HDV is less common but has been reported in Southeast and Southwest Asia. It is often associated with HBV genotype D, sporadically found among migrants from regions where genotype D is more prevalent. The clinical implications and disease progression of HDV genotype 2 in the Asian population require a more comprehensive investigation (Su et al., 2006).

The genotypes of HDV in Southeast Asia play a significant role in disease progression, treatment response, and clinical outcomes. HDV infection exacerbates the severity of liver disease and increases the risk of developing chronic hepatitis, cirrhosis, and HCC (Sinniah et al., 1986). Genotype-specific differences in pathogenesis, immune response, and treatment response influence the clinical course

of HDV infection among individuals co-infected with HBV (Robinson et al., 2023). Public health interventions in Malaysia should consider the genotypes of HDV when formulating prevention and control strategies. Efforts to control HDV are closely intertwined with those aimed at preventing and managing HBV, as effective control of HBV transmission is crucial to reducing the risk of HDV co-infection (zhi-Lim et al., 2022). Strategies to enhance HBV vaccination coverage, promote regular screening for both HBV and HDV, and improve access to antiviral therapies are essential for effectively managing HDV infection in Malaysia (zhi-Lim et al., 2022).

Genotyping of HDV-infected individuals can provide valuable insights into the genetic diversity and transmission patterns of HDV in Malaysia. It can aid in understanding the prevalence of different genotypes and their association with disease severity, treatment response, and prognosis. Genotypic characterization of HDV strains can guide treatment decisions, such as the selection of antiviral therapies, as different genotypes may exhibit varied sensitivity to antiviral agents (Tan et al., 1989). The genetic diversity of HBV genotypes in Asia contributes to disease progression, treatment response, and vaccine effectiveness variations. Genotypes, such as genotype C, are associated with a higher risk of chronic hepatitis B, cirrhosis, and HCC development. Genotype-specific differences in viral replication, immune response, and pathogenesis influence the clinical outcomes observed among different populations (Bello et al., 2023).

Asian public health interventions must consider the prevalent genotypes when implementing prevention and control measures. Vaccination programs targeting specific genotypes can be tailored to optimize protection, especially in regions with a

predominant genotype. Additionally, genotyping of HBV-infected individuals can guide treatment decisions, as specific genotypes may exhibit different drug resistance patterns (Rajamoorthy et al., 2020). The emergence of HBV drug resistance poses a significant challenge to the effectiveness of antiviral treatment. Drug resistance occurs when the virus mutates, rendering it less susceptible or resistant to the effects of antiviral medications (Cheang et al., 2013).

HBV drug resistance typically arises due to genetic mutations in the viral genome, particularly in the region encoding the viral polymerase, which targets most antiviral drugs. These mutations can alter the structure and function of the viral polymerase, reducing its susceptibility to antiviral agents and allowing the virus to replicate and persist despite treatment (Suppiah et al., 2014). There are two primary mechanisms of HBV drug resistance (Di-Lello et al., 2019; Mokaya et al., 2021): Primary and secondary drug resistance mechanisms. Primary drug resistance refers to drug-resistant HBV strains in individuals who have never received antiviral therapy. These resistant strains can be transmitted from an infected individual or can arise through de novo mutations during the early stages of infection. Primary drug resistance can significantly limit treatment options and compromise the effectiveness of initial therapy (Suppiah et al., 2014). Secondary drug resistance occurs during or after antiviral therapy. It is characterized by the emergence of drug-resistant HBV strains in patients who were previously responsive to treatment. Secondary drug resistance typically arises due to the selective pressure exerted by antiviral drugs, leading to the outgrowth of HBV variants with mutations conferring resistance to specific medications (Di-Lello et al., 2019). Managing HBV drug resistance poses several challenges for healthcare providers and researchers. These challenges include reliable

methods to detect drug resistance, access to alternative antiviral agents, and developing strategies to prevent resistance emergence (Mokaya et al., 2021).

1.2 Justification

There is a high demand for genotype information and investigation regarding HBV and HDV-infected individuals. The available data on the incidence of the Hepatitis B virus in Malaysia has been on an upthrust trend over the last decade (Hudu et al., 2018). Aside from the significant impact HBV and HDV have on global health, the molecular epidemiology of HBV and HDV within Malaysia is essential due to the varying clinical profiles associated with the different genotypes of the virus. The natural clinical history of hepatitis is usually altered with Hepatitis D virus co-infection, rendering established antiviral therapies ineffective (Koh et al., 2019). Furthermore, an improved understanding of the distribution of the different genotypes of HBV and HDV, the dynamism of HBV antiviral resistance, and the clinical significance of different genotypes and risk factors could help physicians develop effective therapeutic strategies on the control of HBV and HDV in Malaysia and entire Asia.

Hepatitis B virus (HBV) and Hepatitis D virus (HDV) infections pose significant public health challenges in Malaysia, substantially impacting the population and healthcare system (Ahmad et al., 2016; Muhammad et al., 2023; Shafie et al., 2018; Yap, 1994). These viral infections primarily affect the liver and can lead to severe health consequences, including chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Malaysia has been working diligently to prevent and

manage both HBV and HDV infections, recognizing the importance of comprehensive strategies to control transmission and improve healthcare services.

HDV is a unique and highly pathogenic virus that requires HBV co-infection to cause disease. The transmission of HDV follows similar routes as HBV, primarily through percutaneous exposure to infected blood or sexual contact (Candotti et al., 2017; Sellier et al., 2018; Veronese et al., 2021). Co-infection with HDV aggravates the severity of liver disease and increases the risk of developing chronic liver inflammation, cirrhosis, and HCC. Efforts to control HDV in Malaysia are closely intertwined with those aimed at preventing and managing HBV, as effective control of HBV transmission is crucial to reducing the risk of HDV co-infection.

Challenges persist while Malaysia has made significant strides in combating HBV and HDV. Limited awareness and understanding of the infections, particularly in rural areas, contribute to delayed diagnosis and limited access to appropriate medical care. Inadequate surveillance systems and limited availability of screening and diagnostic facilities further hinder the effective identification and management of cases (zhi-Lim et al., 2022). There are diverse populations with varying ethnic groups and cultural practices that influence the transmission dynamics of HBV and HDV in Malaysia. A prior molecular study has revealed the presence of multiple HBV genotypes and sub-genotypes circulating in Kuala Lumpur, the national capital, with varying distribution patterns across different regions and ethnic communities (Hudu et al., 2018). The genetic diversity of HBV has implications for disease progression, treatment response, and vaccine effectiveness. Understanding the molecular characteristics of HBV strains circulating in Malaysia is essential for tailoring

prevention and treatment strategies to the specific viral variants prevalent in different populations (Hudu et al., 2015).

1.2.1 Conceptual framework

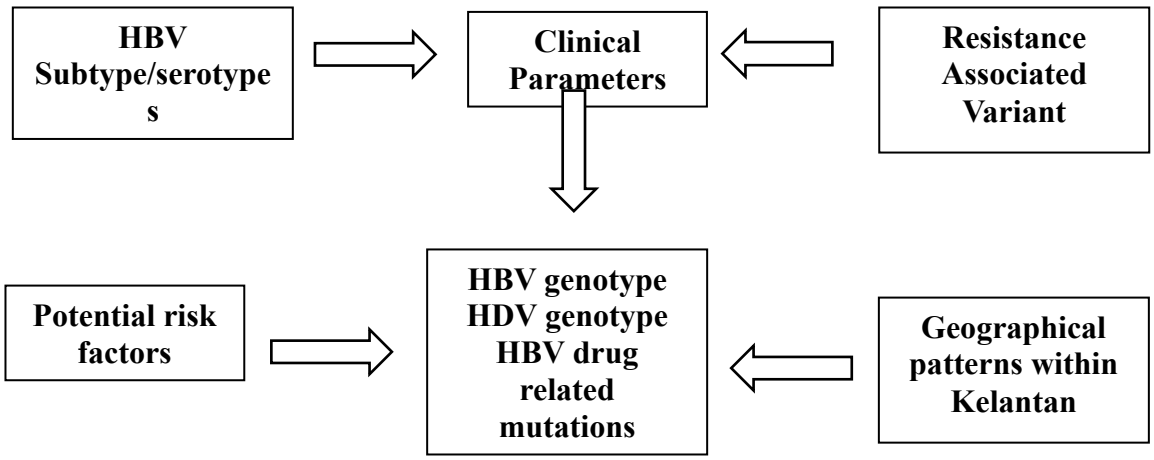

Figure 1.1 Conceptual frame work

1.3 Research hypotheses

1. Ho: There is diverse Hepatitis B virus genotype within circulation in the study population.

   Hi: There is no diversity in HBV genotypes within the study population

2. Ho: There is diverse Hepatitis D virus genotype within circulation in the study population.

   Hi: There is no diversity in HDV genotypes within the study population

3. Ho: There is no presence of antiviral resistance within the study cohort

   Hi: There is presence of antiviral resistance within the study cohort

1.4 Research questions

1. What are the genotypes of HBV in circulation within the study population?
2. What is the molecular evolutionary relatedness between the existing genotypes of HBV circulation within the study population?
3. What is the frequency of Hepatitis D virus infection among Chronic Hepatitis B patients?
4. What are the antiviral resistance genes of the Hepatitis B virus within the study population?

1.5 Research Aim and Objectives

1. General objective

This study aims to determine the molecular epidemiology of HBV and HDV in Kelantan, Malaysia.

2. The specific objectives of the study are to determine:
    a. Genotypes of HBV and HDV,
    b. HBV and HDV genomic variability and evolutionary relatedness,
    c. HBV resistance to selected antiviral therapies and,
    d. Associated risk factors regarding HBV and HDV infections within the study population.

CHAPTER 2

LITERATURE REVIEW

2.1 Hepatitis B Virus (HBV)

Hepatitis B virus (HBV) is a hepadnavirus liver viral infection that causes liver inflammation and can lead to severe medical complications (Summers et al., 2003). Cirrhosis of the liver, hepatocellular cancer, and acute or chronic hepatitis can all result from HBV infection (Bello et al., 2023). The morbidity and incidence of HBV are still significant globally; approximately, there are about 360 million chronic HBV infections worldwide, and the virus has been exposed to over 2 billion people. The illness is responsible for 600,000 annual fatalities worldwide, so HBV is a significant threat to world health (Locarnini et al., 2015).

The effects of the virus are not immediately cytotoxic. The host immune system's activation is thought to be what damages the liver (Kennedy et al., 2012; Locarnini et al., 2015). Viral multiplication and immune system defenses interact unfavorably when a virus enters the body. The virus is forced to adapt by immune system assaults. Due to HBV's prone to error replication process, evolution may proceed if these altered strains/virions are picked and transmitted to a new host or another person (Kennedy et al., 2012). The origins and precise genealogy of HBV have yet to be discovered. Due to genomic variety and human migration, HBV has evolved into genetically diverse clusters, or genotypes, each with a unique geographic distribution. By examining the relationships between the strains that have been gathered, phylogenetic analysis can be utilized to follow the genetic footprints of these

genotypes. These traces are gradually becoming obsolete in an era of globalization and frequent travel (Feng et al., 2020).

2.2 HBV structure

The hepatitis B virus belongs to the Hepadnaviridae family. The two genera that make up this virus are Avihepadnaviruses and Orthohepadnaviruses, which both infect birds and mammals, respectively (Jilbert et al., 1996). Aviahepadnaviruses have been discovered to be carried by Storks, ducks, herons, and flamingoes. The HBV viruses of mammals and avians have highly diverse genomic, structural, and morphological characteristics (Jilbert et al., 1996).

The HBV genome comprises a 3200-base pair, circular, partially double-stranded DNA (Mo & XiangYang, 2014; Yu et al., 2005). The entire viral unit (virion), commonly known as the "Dane particle", has a diameter of 45 nm (figure 1). Three envelope proteins are covered by a lipid bilayer in an icosahedral capsid (Zhang et al., 2015). The three Hepatitis B surface antigens (HBsAg) molecules have different start codons, yet they are all translated from the same reading frame. The size, weight, and nucleo-protein glycosylation are made in ratios of 4:1:1 and referred to as SHBs (small), MHBs (medium), and LHBs (large), respectively (Bert et al., 2020; Zhang et al., 2015).

The reverse transcription polymerase is present in the nucleo-capsid, which is pre-originated by the Hepatitis B core protein (HBcAg) (Bert et al., 2020). The representative structure of the hepatitis B virus is represented in Figure 1. The DNA

genome is connected to transcriptase activity and RNA polymerase H. HBV-infected cells also secrete protein X from the core of the nucleocapsid, which has unclear regulatory functions. The e antigen (HBeAg), a serum-based protein with significant immune-regulating properties, is also secreted in the nucleocapsid core (Bert et al., 2019).

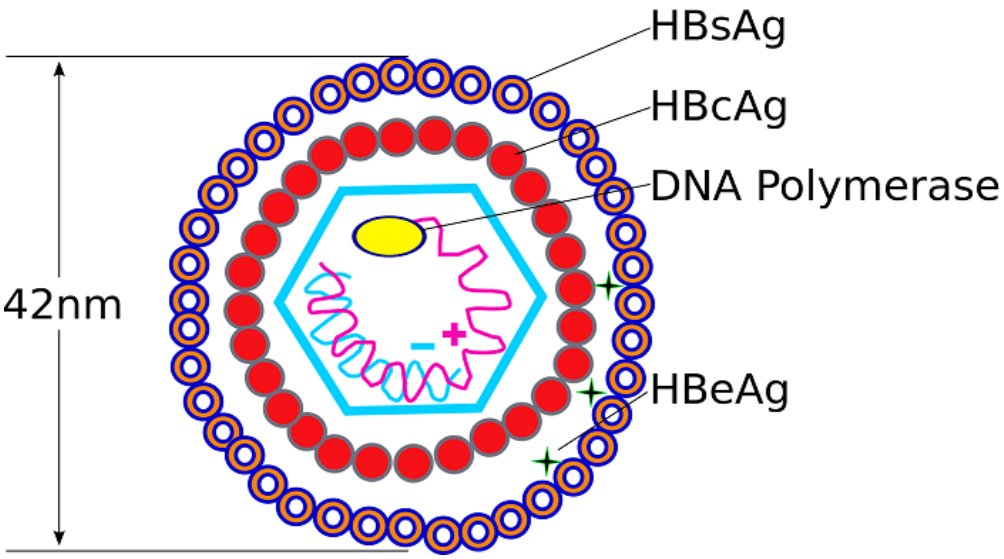

Figure 2.1: Structure of HBV

This figure was adapted from Liang (2009) (Liang, 2009).

Figure 2.1 Structure of HBV

2.3 Epidemiology of Hepatitis B virus

According to Mak et al. (2020), the incidence and morbidity of chronic HBV differ worldwide, from over 11% in some countries in Asia and Africa to less than 5% in the Northern parts of Europe (Mak et al., 2020). Smith et al. (2017) claim that Sweden has a low endemic prevalence of HBV infections. The Swedish Institute for Infectious Disease Control monitors the epidemiological landscape and obtains information on each formally proven HBV case (Nilsson et al., 2019).

A prevalence of less than 0.5 percent and an incidence of 12 to 21 new cases of HBV per 100,000 people are recorded yearly in Sweden (Nilsson et al., 2019). Approximately 200 cases of acute infections are reported each year (Figure 2), with many of these infections occurring as a result of sexual contact or drug use involving injection (IDU) (Abdala et al., 2008), with the majority of reported cases being chronic infections in immigrants who have recently arrived from endemic regions. Nevertheless, this group has also been found to have additional genotypes.

In Sweden and the rest of Northern Europe, genotypes A2 and D3 have been most common among patients with acute HBV infection; nevertheless, increased travel has helped other genotypes proliferate. In the county of Stockholm, there were 154 instances of acute hepatitis recorded between 2004 and 2007 (Nilsson et al., 2019). Of those cases, 65, 25, and 18 belonged to genotypes D3, D1, and C2, respectively. The rise in the prevalence of sub-genotypes A2 and D1 is speculative of an increased sexual transmission as homosexual men are more likely to carry A2 than heterosexual women are to carry D1 (Zehender et al., 2012).

In Asia, the toll of Hepatitis B infection is still of public health concern; according to a systematic review of HBV genotypes in Asia, HBV genotype C has the highest prevalence, followed by HBV genotype B (Bello et al., 2023). There is still a high toll of HBV incidence within Southeast Asia (André, 2000; Poss, 1989; Wait et al., 2016). According to WHO, countries like Singapore, Cambodia, Vietnam, and Thailand still have a high incidence of HBV (Chudy et al., 2012; WHO, 2010). The epidemiology of HBV in Malaysia has reduced significantly since the introduction of the child vaccination program by the Malaysia government; a recent study of the incidence of vaccine escape HBV among blood donors in Malaysia reveals a new pattern of disease transmission and suggests the emergence of a highly infectious vaccine escape HBV mutant among the Malaysian population (Hudu et al., 2013, 2015; Zhi-Lim et al., 2022)

The incidence of HBV in Africa is still on the rise despite the available preventive measures and awareness. Africa still accounts for a significant toll of HBV globally; a recent study of a Nigerian cohort reveals a high toll of HBV incidence in the most populous black nation in the world (Ifeorah et al., 2017). The pattern of transmission of the disease in Africa has been influenced by travel and history of multiple sexual partners and is more prominent in intravenous drug users (Bialek et al., 2005; Torbenson et al., 2004).

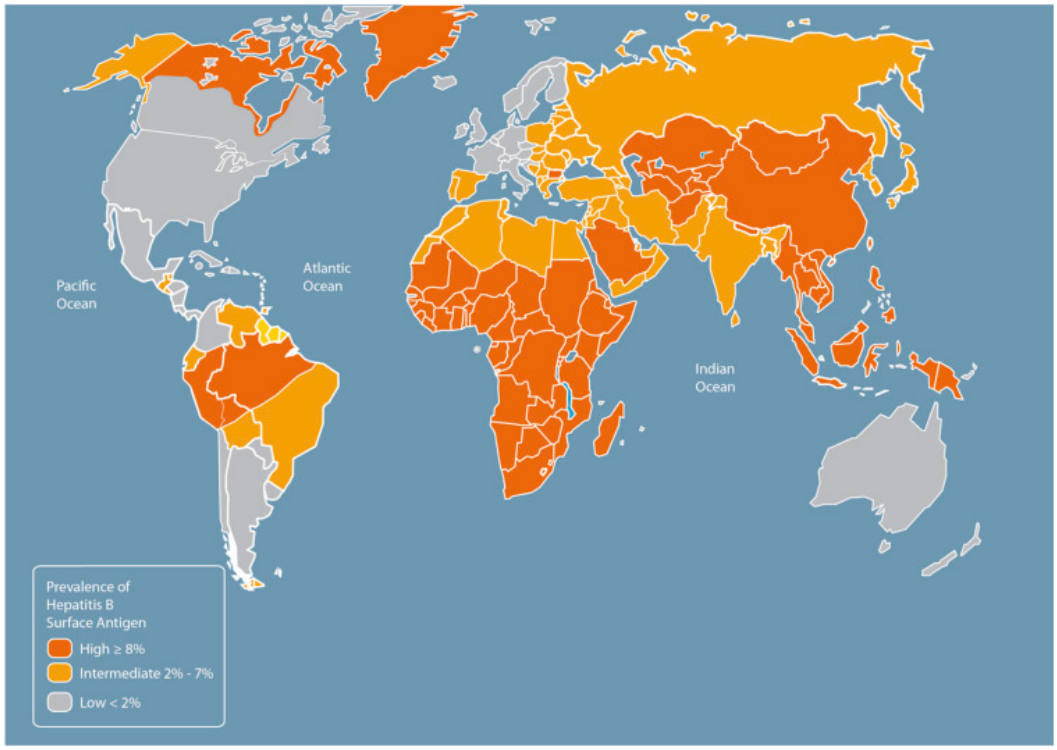

Figure 2.2 Global epidemiology of HBV

This figure was adapted from Pedersini et al. (2016) (Pedersini et al., 2016).

2.3.1 HBV genotypes and subtypes

HBV genotypes have been categorized into eight (A–H) based on an amino acid and nucleotide difference of more than 8%, representing their origins in geography and ethnicity (Kafeero et al., 2022). Genotype A predominates in Africa, Europe, and South America. In contrast, genotypes B and C dominate South and East Asia, genotype D is predominant in the Middle East and the Mediterranean, genotype E is indigenous to Western Africa, and genotypes F and H predominate in America (Shiina et al., 2019). Clades within these sub-genotypes that have less than 4% diversity are represented by phylogenetic clustering. Recently, there has been a discussion on the standards used to define genotypes and subcategories and the pursuit of uniform standards. For genotypes to fit more closely into phylogenetic groups, a nucleotide difference of not more than 7.49% based on the entire genomic sequence has been proposed (Lim et al., 2021). It has been suggested that the genotype X/C recombinant, first detected in individuals of Vietnamese descent resident in Sweden, should be classed as genotype "I" in addition to these recognized genotypes (Velkov et al., 2018).

However, the low genetic and substitution diversity to the nearest genotype (Diarra et al., 2018) has disputed whether it should be classified as a novel or recombinant genotype. Recently, a patient in Borneo of Japanese ethnicity was found to have a new HBV strain (JRB34) that differed from known genotypes by more than 10% and showed no signs of recombination. The novel recombinant was suggested to be denoted as genotype "J" designation. Still, interestingly, phylogenetic analysis of its entire genomic sequence showed that it had more in common with HBV from apes than with human HBV genotypes (Feng et al., 2018).

Genotype A is commonly found in South Asia, including India and Pakistan. It is also prevalent in Central Asia, such as Uzbekistan and Tajikistan. Genotype A is associated with a higher risk of vertical transmission and an increased likelihood of developing hepatocellular carcinoma (HCC) (Bello et al., 2023; Harris et al., 2017; Noverati et al., 2022). Genotype B is prevalent in East Asia, particularly in China, Taiwan, and Korea. It is also found in Southeast Asian countries like Vietnam and Thailand. Genotype B is associated with a higher rate of Hepatitis E antigen (HBeAg) positivity, indicating higher viral replication and an increased risk of chronic hepatitis B development (Bello et al., 2023; Lee et al., 2017).

Genotype C is widespread in East Asia, including China, Korea, and Japan. It is also prevalent in Southeast Asian countries such as Vietnam and Thailand. Genotype C is associated with a higher risk of developing chronic hepatitis B, progression to cirrhosis, and an increased incidence of HCC (Bello et al., 2023; Komatsu et al., 2015; Nun-Anan et al., 2015). Genotype D is prevalent in South Asia, including Pakistan and Afghanistan. It is also found in parts of Central Asia. Genotype D is associated with more severe liver disease outcomes, including a higher risk of liver cirrhosis and HCC (Bello et al., 2023; Ho et al., 2022). Genotype E is primarily found in West Africa but is also observed in parts of Central Asia, such as Kazakhstan and Uzbekistan. Its presence in Asia may be attributed to migration patterns (Suijkerbuijk et al., 2018).

Genotype F is primarily found in Central and South America but has been sporadically reported in parts of East Asia, such as Japan and China. The significance of genotype F in Asia has yet to be fully understood and requires further investigation

(Araujo et al., 2013). Genotype G is predominantly found in North and Central America, but occasional cases have been reported in Southeast Asia, including Vietnam and Thailand. Like genotype F, the prevalence and clinical implications of genotype G in Asia warrant further research (Araujo et al., 2013).

2.3.2 HBV serotypes

Before genotypes were identified, HBV strains were traditionally divided into nine classes based on the antigenic and serological characteristics of the HBV surface protein (Pfefferkorn et al., 2018). These classes were: adw2, adw4q-, adrq+, adrq-, ayw1, ayw2, ayw3, ayw4 and ayr. This classification's molecular foundation was variance at a few S region locations. All serotypes share the key antigenic determinant known as the determinant (aa 124–148) (Chaar et al., 2010). The Lys/Arg substitutions at residues 122 and 160 cause the d/y and w/r variants. The mutation at residues 177 and 178 is likely the final factor contributing to q type. Genotypes and subgenotypes have largely supplanted serotypes (Thijssen et al., 2020).

2.3.3 HBV genotype recombination

Genetic sequences and viral characteristics may be altered through recombination. Though the interchange of nucleic acid segments from two coexisting viruses may often occur in HBV, the scant number of recombinants discovered suggests that few of these recombinants have selective advantages (Ko et al., 2021). Recombination may go unnoticed (and thus go unreported) because it frequently requires specialized testing with Simplot analysis or other similar techniques to discover (Kafeero et al., 2022).

Sequences of genotype B contained core regions more comparable to genotype C. Five HBV strains with unusual RFLP genotyping patterns were studied earlier (Araujo et al., 2020). According to Simplot and bootstrapping, there was a high level of similarity between genotype C and genotype A at nucleotide position between 1800 and 2864; the recombinant status was named genotype C/A, and the latter was later reclassified as novel genotype I based on genomic relatedness (Castelhano et al., 2017).

The recombination with genotype C origins for the pre-core and core areas, present in sub-genotypes B (B2-B5, B7) found in Asia, is the most pervasive (Sandhu et al., 2020). Because genotype B strains lack the recombinant propensity and only persist in the Arctic and Japan, it suggests that this recombination has a benefit (sub-genotypes B1 and B6). According to reports, individuals with genotype B2 had worse clinical outcomes than those with genotype B1 (Charlton et al., 2020). The latter may be due to a genotype C core segment. Recombination necessitates the co-existence of virions with strains of diverse genotypes. Hence, recombinants are in an area with various genotypic distributions in circulation (Feng et al., 2018). It is unknown if these co-infections' simultaneous or sequential transmission results in their establishment. The ability of recombinants to spread is likely influenced by both the population's sensitivity and their fitness (Wang et al., 2005).

Some recombinants have gained popularity, including the previously mentioned subtype and recombinant found in Tibet (B subtypes and the C/D recombinant). Intergenotypic and intragenotypic recombination has been suggested to be a significant source of genetic variation crucial to the transformation of HBV (Cui

et al., 2002). However, it is relatively restricted, given that the proportion of named recombinants is low compared to point nucleotide mutation. Such recombination arose from multiple circles of genomic evolution (Q. Liu et al., 2016). Inter and intra-species recombination are likewise conceivable. There is empirical proof of a chimpanzee-HBV isolated from East Africa that contained about 500 base pairs of HBV genotype C nucleotide unit (Chang et al., 2017). However, this is likely an uncommon occurrence because no other cases have been documented. Genotype G is another instance of potential interspecies recombination and may result from recombination between extinct genotypes' ancestral strains (Hoan et al., 2021).

2.4 Replication of HBV

HBV is a DNA virus with reverse transcriptase that disseminates via intermediary RNA. Even though the precise process by which HBV enters the hepatocyte is still unknown, it is most likely through the preS region in the HBsAg (Summers et al., 2003). This receptor-ligand interaction might explain the high selectivity of hepatocytes. A similar pattern was also observed in the Kidney, liver, and lymphocytes. An incomplete DNA strand is transported to the core of the nucleic strata of the virus (nucleus), where it is completed by the capsid, which enters the cell (Warren et al., 2006).

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liver, and lymphocytes. An incomplete DNA strand is transported to the core of the nucleic strata of the virus (nucleus), where it is completed by the capsid, which enters the cell (Warren et al., 2006).

Once HBV gains access to the hepatocyte, the virus undergoes uncoating, a critical step in the replication cycle. Uncoating refers to the process by which the viral capsid is disassembled, releasing the partially double-stranded viral DNA into the host cell's nucleus. The uncoating process allows the viral genome to reach its replication site and facilitates the initiation of the viral life cycle. The exact mechanisms underlying HBV entry and uncoating still need to be fully elucidated. However, several cellular factors and viral components have been implicated in these processes. Identifying specific receptors involved in HBV attachment, internalization, and uncoating has provided valuable insights into the early stages of infection. Additionally, recent advancements in imaging techniques have allowed for the visualization and characterization of the dynamic interactions between HBV and host cells during entry and uncoating (Zhang et al., 2015).

The viral DNA serves as a template for synthesizing viral RNA transcripts within the infected hepatocyte's nucleus. This transcription process is mediated by the host RNA polymerase II, a key enzyme responsible for transcribing protein-coding genes in eukaryotic cells. The HBV genome contains four overlapping open reading frames (ORFs) known as preC/C, preS/S, preS2/S2, and X (Summers et al., 2003). One of the most critical transcripts produced during HBV replication is the pregenomic RNA (pgRNA). The pgRNA is transcribed from the preC/C ORF and serves as a viral replication and translation template. It is unique among HBV RNA transcripts because