Open Access Article

A DETAILED REVIEW ON HEPATITIS B VIRUS (HBV)

Reshmi T Nair
Ph.D. Scholar, Jaipur Engineering College and Research Centre University, Jaipur, Rajasthan, India.
reshmitnair19@gmail.com

Bharti Malhotra*
Senior Professor, Department of Microbiology, SMS Medical College & Hospital, Jawahar Lal Nehru Marg, Jaipur 302004, India. drbhartimalhotra@gmail.com

Sonali Pandey
HOD Department of Botany, Jaipur Engineering College and Research Centre University, Jaipur, Rajasthan, India, drsonali17@gmail.com

ABSTRACT
Hepatitis B virus infection has become a major public health problem worldwide, approximately 30% of the world's population show serological evidence of current or past infection. Inflammation of the liver, vomiting, jaundice, and mortality are all symptoms of the acute sickness. Chronic hepatitis impact to global morbidity and mortality is typically underestimated because it is a "silent" disease. Both the incidence of new infections and the risk of chronic liver disease must be reduced as part of hepatitis B and C prevention and control. The prevention and detection of HBV and HCV infections should be done by comprehensive public health prevention program. HBV infection can be prevented by taking universal precautions in health-care settings, such as avoiding needle-stick injuries and instituting post-exposure prophylaxis. The present study focuses on the effects of vaccination and also a detailed review has been conducted over the HBV genotype/genetic characterization and HBV mutation/genetic variability.

Keywords: Hepatitis B virus, HBV genotype, Vaccination, HBV Awarness, Serological markers

抽象的

乙型肝炎病毒感染已成为世界范围内的主要公共卫生问题，世界上大约 30% **的人口**显示出当前或过去感染的血清学证据。肝脏发炎、呕吐、黄疸和死亡都是急性疾病的症状。慢性肝炎对全球发病率和死亡率的影响通常被低估，因为它是一种**“无声”疾病。作**为乙型和丙型肝炎预防和控制的一部分，必须降低新感染的发生率和慢性肝病的风险。 HBV**和**HCV**感染的**预防和检测应通过全面的公共卫生预防计划来完成。 HBV **感染可以通**过在医疗机构中采取普遍的预防措施来预防，例如避免针刺伤和制定暴露后预防措施。本研究侧重于疫苗接种的影响，并对 HBV **基因型**/遗传特征和 HBV **突**变/遗传变异性进行了详细审查。

Received: October 05, 2021 / Revised: October 31, 2021 / Accepted: November 30, 2021 / Published: December 24, 2021

About the authors：Bharti Malhotra

Corresponding author- *Email: drbhartimalhotra@gmail.com

关键词：乙型肝炎病毒，HBV基因型，疫苗接种，HBV知晓，血清学标志物

1. INTRODUCTION

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae family, having 3.2kb largely double-stranded relaxed circular DNA genome and a compact coding organization (Seeger et al., 2013). In the year 2020 WHO has reported that Hepatitis B virus (HBV) has infected one-third of the world's inhabitants, resulting in 257 million chronic infections and over 880,000 deaths every year, the most of the causalities are due to cirrhosis and hepatocellular cancer (HCC).Hence, Hepatitis B virus infection has become a major public health problem worldwide, approximately 30% of the world's population show serological evidence of current or past infection. Hepatitis B virus can be detected by several serological markers as per stage of disease: HBsAg and anti-HBs, HBeAg and anti-HBe, and anti-HBc IgM and IgG. It is transmitted through contact with infected blood and semen.

Phylogenetically HBV has been divided into nine genotypes (A–I) and one suspected genotype based on >7.5 percent genomic sequence difference (J). The substantial variation within distinct HBV genotypes has led to sub-genotyping into a plethora of sub-genotypes (Kramvis, 2014). Although, the geographic distributions of HBV genotypes and sub-genotypes differ,HBV has another replication cycle that comprises an error-prone reverse transcriptase (RT) which results in a variety of viral variants more precisely quasispecies. Constant HBV evolution leads to the selection of mutations by endogenous (host immune system) and exogenous (antiviral therapy and immunisation) mechanisms, energizing a molecular arms race between the virus and the host, ultimately resulting in the appearance of mutations involved in a variety of clinical outcomes (Araujo et al., 2011; Rajoriya et al., 2017; Revill et al., 2020).

Inflammation of the liver, vomiting, jaundice, and mortality are all symptoms of the acute sickness. Chronic hepatitis B can lead to liver cirrhosis and cancer, both of which are deadly diseases with inadequate treatment options. The age of the afflicted person has a big impact on whether the condition becomes chronic or goes away completely. Approximately 90% of infants who are infected at birth will develop chronic illness. However, the likelihood of chronic infection diminishes with age. Among Indians, chronic hepatitis B virus (HBV) infection is still a major public health concern. However, there is growing evidence that progression of the disease depends on the infecting genotype, patients with genotype C showing a more severe evolution of liver disease compared with those with genotype B (Chu et al., 2006).

Antiviral therapy with nucleos(t)ide analogues (NAs) is a good control measure, although it takes a long time to work. During long-term treatment, the limited number of NAs accessible and the development of drug resistance due to viral changes in the HBV reverse transcriptase (rt) domain are a serious issue as it leadsto treatment failure. Antiviral resistance has been found in HBV isolates among therapy-naive patients, according to recent publications. (Ntziora et al., 2009, Pastor et al., 2009, Solmone et al., 2009).Although none of the available treatments can cure the infection, they can prevent it from spreading and causing liver

damage. Nucleoside analogues (lamivudine, Telbivudine, and Entecavir), nucleotide analogues (Adefovir, Tenofovir, and Emtricitabine), and pegylated interferon alpha-2a or alpha 2b once weekly are the seven drugs now approved for the treatment of hepatitis B infection. NA primarily inhibits the manufacture of reverse transcriptase (RT), which is required for viral replication, and hence decreases HBV replication and reduces hepatocyte damage. The most significant disadvantage of NA is the return of HBV following a break in treatment. HBV polymerase reverse transcription is inhibited by NA. Because nearly all cases of NA resistance are initially diagnosed by a sustained elevation in viral load that occurs despite continuous antiviral medication, measuring viral load is essential for monitoring and verifying the presence of drug-resistant virus (Locarnini and Mason 2006). Conformational alterations caused by mutations can impact the binding of neutralising antibodies produced during natural infection or after active or passive vaccination (Zuckerman and Zuckerman, 2003).

Several possible outcomes of immune escape mutations include false-negative results from commercial HBsAg assays (occult hepatitis B), evasion of anti-HBV immunoglobulin therapy, and vaccine-induced immunity. G145R was the first escape mutation discovered and is the most common HBV variation in people with demonstrated vaccination escape capabilities (Carman et al., 1990). Around the years, a number of additional mutations linked to immune evasion have been discovered all over the world (Cooreman et al., 2001; Lazarevic et al., 2019). HBV RT activity is inhibited by nucleos(t)ide analogues (NAs). So far, six NAs have been licenced for the treatment of chronic HBV infections: lamivudine (LAM), telbivudine (LdT), adefovir dipivoxil (ADV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) (Sarin et al., 2016; Easl, 2017; Terrault et al., 2018). Drug-resistant mutations, on the other hand, frequently occur during long-term usage of low-barrier-to-resistance medicines like LAM, resulting in treatment failure and progression to liver disease. (Zoulim, 2011). Furthermore, because the HBV genome has overlapping reading frames, changes in the polymerase gene caused by antiviral selection pressure may impact neutralisation epitopes within HBsAg. (Pollicino et al., 2009).

Prevalence

Hepatitis B virus infection is a major public health issue around the world, with approximately 257 million people infected. In the year 2015, it was predicted that 887,000 people died worldwide as a result of consequences from chronic HB virus infection (WHO, 2017).HBV infection is widespread worldwide and unevenly distributed, with three resultant geographic categories to describe endemicity: (i) areas of highendemicity (>8%) characterizing mainly developing countries (Sub-Saharan Africa, South East, and Far East Asia),

(ii) areas of intermediate endemicity (2-7%) which coverthe Mediterranean, Eastern Europe, and Latin America, and(iii) areas of low endemicity (<2%) represented by Western Europe, North America, and Japan (Liaw and Chu, 2009). India's chronic HB virus infection rate is 1.46 %, with an estimated 17 million chronic carriers, according to a meta-analysis of published data (Schweitzer et al., 2015).In the WHO EasternMediterranean Region, an estimated3.3% of the general population is infected (Kao and Chen, 2018). Global Health Sector Strategy which advocated for viral

hepatitis in 2016 to be eradicated as a public health issue by 2030, the World Health Assembly supported this strategy. (WHO, 2016).
The modes of transmission vary slightly from one country to another, due to the differencesin the blood transfusion safety protocols and preventive measures implemented by the governments. Meanwhile, HBVtransmission is predominantly horizontal, resulting fromthe exposure of abraded skin, cuts, minor open wounds, ormucosal surfaces to blood or body fluids containing HBVfrom the afflicted subjects (Komatsu et al, 2016).

SIGNIFICANCE ON PUBLIC HEALTH/CHRONIC HBV

Chronic hepatitis B is primarily transmitted horizontally in early childhood (mainly through family interactions) and to a lesser extent perinatally in India. Horizontal transmission is unknown, however it could be caused by non-intact skin or mucous membranes coming into touch with tears, saliva, or blood carrying HBV-infected fluids, or by sharing toothbrushes (Ray, 2017). The other mode of transmission is parenteral transmission, which can occur at any age (for example, infected blood or blood products transfusion, intravenous drug use, unsafe therapeutic injections, occupational injuries, or nosocomial (hospital-acquired) transmission during healthcare-related procedures such as surgery, haemodialysis and organ transplantation (WHO, 2021).

Chronic hepatitis impact to global morbidity and mortality is typically underestimated because it is a "silent" disease. Both the incidence of new infections and the risk of chronic liver disease must be reduced as part of hepatitis B and C prevention and control. The prevention and detection of HBV and HCV infections should be done by comprehensive public health prevention program. The infection diagnosis and control of viral hepatitis-related chronic liver disease, surveillance and monitoring of prevention activities' effectiveness, and the establishment of a research agenda must be done. (Lavanchy et al., 2008).

Hepatitis B and liver cancer

Last-stage liver disease accounts for almost one in forty deaths worldwide. Chronic liver infections with hepatitis B virus and hepatitis C virus (HCV) are well-recognized risk factors for cirrhosis and liver cancer, but estimation of their contribution to worldwide disease burden have been lacking (Joseph F.Perz et. al). Diverse roles of hepatitis B virus in liver cancer has been studied by Guillaume Fallot, according to the study hepatitis B burden is particularly heavy in endemic countries, where liver cirrhosis and HCC are leading causes of death. However, the oncogenic character of HBV remains enigmatic. HBV virus has no cytopathic effect; hence, liver damage is attributed to immune responses that induce apoptosis, inflammation and regeneration. Frequent integration of HBV DNA into host chromosomes may result into insertional mutagenesis of cancer-related genes and chromosomal instability. HBV proteins, notably the HBxtransactivator, participate as co-factors in oncogenesis. For improving disease management better understanding of hepatitis B pathogenesis is mandatory.

HBV is found to be the second most prevalent cause of chronic liver disease (CLD) in India. However, liver cancer is being attributed to growing alcohol consumption rates these days

rather than true HBV infection prevalence, which has stayed most prevalent cause since long. Worldwide approximately 350 million people are chronic carriers of HBV. The infection causes acute and chronic liver disease including hepatocellular carcinoma (HCC) and cirrhosis. The natural history of chronic infection based on virus-host interactions can be divided into three phases, immune tolerance, immune clearance, and viral integration phases. Hepatocellular carcinoma (HCC) is still most commonly caused by HBV in India; it has been found to be linked to 43% of HCC cases, across India. Even with HCC at the first clinical presentation in India, a considerable proportion of patients present in the advanced stages of the disease, when curative therapy is not viable (Ueno et al., 2017). Xiaodong Zhang et. al studied theEffects of hepatitis B virus X protein on the development of liver cancer, study suggests that the HBV X protein (HBx) plays a crucial role in the pathogenesis of HCC. The high occurrence of anti-HBx antibody in the serum of HCC patients indicates that it could be a prognostic marker of HBV infection and HCC.

High-risk patients of HBV (i.e. sexual and household members in close contact with patients/carriers, health care workers, dialysis patients, intravenous drug users, persons who receive multiple blood transfusions, participate in acupuncture, are incarcerated, or on immune suppressives, biologics, or cancer chemotherapy, etc.) should be screened and vaccinated on a regular basis. In order to avoid unnecessary injections and implement safe injection practises, health education is required, not only for the general public and the high-risk group in particular, but also for health care staff (like use of aseptic technique and disposable syringes or fluid infusion sets for multiple patients, and taking proper precautions when multiple-dose vials are used) (Duong et al., 2015).

Structure of Hepatitis B virus:

The virus particle, (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses (Locarnini 2004). The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. 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 antigen (HBsAg), and is produced in excess during the life cycle of the virus (Howard 1986).

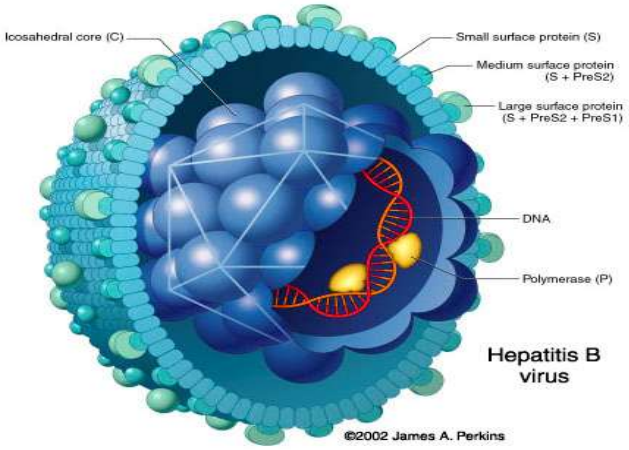

Figure 2.4 An illustration of Hepatitis B Virus (HBV) (©2002 James A. Perkins, Medical and Scientific illustrations, http://people.rit.edu/japfaa/infectious.html)

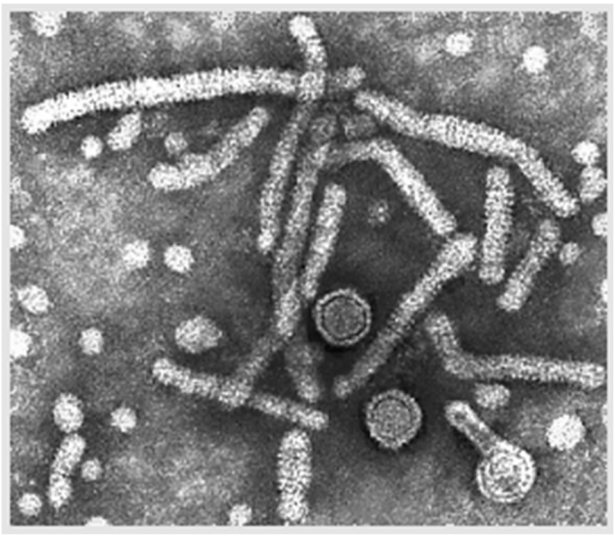

Figure 2.5 Electron micrograph of HBV virions in infected liver tissue (Stannard 1995).

2.3.1 Genome: In HBV genome, there are 3200 nucleotides encoding four main genes which express the viral proteins. Viral proteins consist of the envelope protein, hepatitis B surface antigen (HBsAg); a structural nucleocapsid core protein, hepatitis B core antigen (HBcAg); and asoluble nucleocapsid protein, hepatitis B e antigen (HBeAg) (Seeger *et al.*, 1986). The core antigen (HBcAg) is encoded by the hepatitis B core gene; 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. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). Virus-infected cells secrete HBeAg (Dienstag, 2008). The outer lipid envelope of the virus contains three types of hepatitis B surface antigen (HBsAg). Small (S), medium (M) and large (L) surface antigens, which are encoded by PreS1, PreS2 and S genes, respectively, are the proteins on outer surface (Gerlich, 2013). Virus-infected hepatocytes secrete HBsAg, but no virions (neither empty nor DNA-filled) (Ning *et al.,* 2011). In other words; the surface antigen is secreted as non-infectious subviral particles (Lau and Wright, 1993;Rehermann and Nascimbeni, 2005; Chang and Lewin, 2007;Brouw*et al*., 2009).

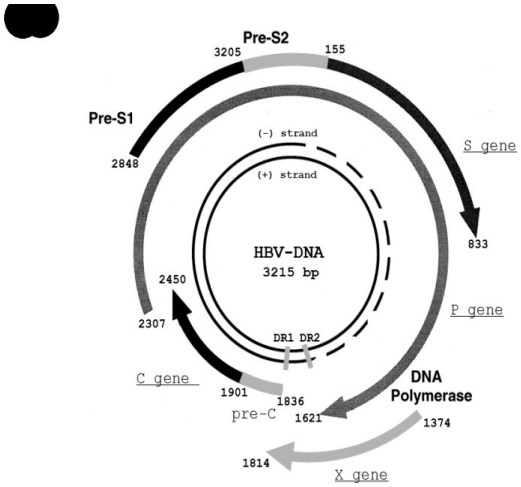

Figure 2.6**Structure and organization of the HBV genome.** The four protein-coding regions are shown by semicircular arrows. They include the precore (pre-C) and core gene (C gene); the polymerase gene (P gene); the X gene; and the envelope genes pre-S1, pre-S2, and S (S gene) (Hsu *et al.,* 1992*).*

2.3.2 Encoding: 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 (Beck and Nassal, 2007).

Serum HBsAg is one of the most significant markers of HBV infection, and the antibodies against the HBsAg indicate the body's immune response to the viral infection. HBeAg is a serum marker of active viral replication when the HBV DNA count in serum is 100,000 to 1 million copies/ml or higher. Hepatitis B endures a retroviral replication which is reverse transcription from RNA to DNA (Seeger *et al.*, 1986). Total destruction of HBV infection is difficult due to the stable, long enduring, covalently closed circular DNA (cccDNA) placed in hepatocyte nuclei and HBV DNA integrated into the host genome (Dienstag, 2008).

2. HBV Genotype/Genetic Characterization

HBV is a gradually evolving virus, several researchers have presented studies to characterize the distribution pattern of HBV genotypes and subgenotypes and HBsAg subtypes in chronic hepatitis B subjects from the Indian subcontinent. The genetic diversity of HBV genotypes also influences the therapeutic response. HBV has been divided into several genotypes based on the genomic sequence. Eight genotypes (A-H) of HBV, in addition with two novel genotypes, I and J, have been discovered. HBV genotypes can be further divided into sub-genotypes, over 30 related sub-genotypes belonging to HBV genotypes have been identified. For genotype, there are > 8% nucleotide differences, and for sub-genotype, there are 4%-8% nucleotide differences. However, the processes behind the various pathogenic characteristics of HBV genotypes are unknown. Several studies have reported that distinct genotypes and sub-genotypes have varying geographic distributions and are linked to disease progression, antiviral therapy response, clinical progression, and prognosis. The A-D and F genotypes are separated into sub-genotypes; the E, G, and H genotypes have no sub-genotypes (Moura et al., 2013; Sunbul, 2014).Genotype A is found in Sub-Saharan Africa, Northern Europe, and Western Africa; genotypes B and C are common in Asia; genotype C is mostly found in Southeast Asia; genotype D is found in Africa, Europe, Mediterranean countries, and India; genotype G is found in France, Germany, and the United States; and genotype H is found in Central and South America. In Vietnam and Laos, genotype I has recently been discovered. In Japan's Ryukyu Islands, the newest HBV genotype, genotype J, has been discovered.HBV genotype distribution could be linked to the route of exposure. Genotypes B and C, are more abundant in high-endemic perinatal or vertical exposure zones, which plays a key role in viral transmission (Liu and Kao, 2013; Lin and Kao, 2015).

The genotype of HBV is essential for a variety of reasons. In patients with CHB, there are clear HBV genotype-related relationships between clinical outcomes and therapy efficacy (Lin et al., 2013). In the year 2000, studies in France found genotype G for the first time;it was discovered in the presence of other genotypes, particularly HBV/A2. Co-infection has also been documented with HBV/C and H genotypes (Sakamoto et al., 2013). The dominance of genotype D and the increased frequency of hepatitis B e antigen (HBeAg)-negative chronic infection are the most noteworthy features of HBV epidemiology in India. There are nine sub-genotypes of genotype D that have been found so far (D1-D9). In chronic HBV patients in Northern India, however, A/D recombinant HBV species have been discovered (Ghosh et al., 2013). Differences in pathogenicity across HBV

genotypes are now partially recognised. Genotypes B and C had higher intracellular levels of HBV DNA and extracellular levels of HBV DNA and HBeAg than genotypes A and D. HBV DNA and viral antigen buildup in hepatocytes may have a role in the development of cellular damage. Furthermore, genotype C's high replication capability may be the cause of increased genotype-related severe liver damage (Kao, 2011).

3. HBV Mutation/Genetic Variability

Hepatitis B virus genetic variability and evolution has been discussed in detail by AlanKay et. al,HBV has been evolving gradually over a long period of time, leading to large amount of genetic diversity, despite the constraints imposed by the complex genetic organization of the viral genome. This genetic diversity is partly due to virus/host interactions and partly due to parallel evolution in geographically distinct areas. Recombination also appears to be an important element in HBV evolution. Karin Kidd-Ljunggren reported **Genetic variability in hepatitis B viruses**HBV genotypes/subgenotypes, as well as HBV genetic variability, are useful in epidemiology and transmission investigations, as well as in tracing human migrations and forecasting the likelihood of severe liver disease and antiviral medication response. Furthermore, knowing the genotype/subgenotype is crucial for developing preventative interventions. As a result, it's critical that new strains are appropriately allocated to their genotype/subgenotype and that nomenclature is uniform, straightforward, and widely accepted (Kramvis, 2014).

The prototype of the Hepadnaviridae family, Hepatitis B virus (HBV), contains a partially double-stranded circular DNA genome of around 3,200 base pairs. This compact genome contains four partly or completely overlapping open reading frames: preC/*C* that encodes for e antigen (HBeAg) and core protein (HBcAg); *P* for polymerase (reverse transcriptase) (POL), *S* for surface proteins [three forms of HBsAg, small (S), middle (M) and large (L)], and *X* for a transcriptional transactivator protein. HBV replicates by reverse transcription of the pregenomic RNA, a 3.5-kb RNA intermediary transcribed from the covalently closed circular form of HBV DNA in the hepatocyte nucleus by the cellular RNA polymerase II. Because POL lacks the ability to proofread, HBV has sequence heterogeneity (Datta et al., 2012; Kramvis, 2014). The mutation rate of the various areas of the HBV genome varies due to the restrictions of overlapping open reading frames and the presence of secondary RNA structures, such as epsilon, coded by nonoverlapping regions (Torres et al., 2013). The HBV genome is thought to change at a range of 10-3 to 10-6 nucleotide substitutions per site per year (Tedder et al, 2013; Andernach et al., 2013; Paraskevis et al., 2013; Bouckaert et al., 2013).HBV has been classified phylogenetically into 9 genotypes, A-I, based on intergroup divergence >7.5 percent across the entire genome (Yu et al., 2010), with a putative 10th genotype, 'J,' isolated from a single individual (Tatematsu et al., 2009). Genotypes A-D, F, H, and I are divided into at least 35 subgenotypes, with an intergroup nucleotide variation of between 4 and 8% throughout the entire genome with excellent bootstrap support.

Table 1. HBV drug resistance reported worldwide

Indian Scientific group	Place	Chemonaive /On Treatment	Genotypes	RT Mutations	Percent	Standard drug(s)
Kandpaland Kuma (2019)	Uttarakhand	On Treatment	A	80V	2.29%	LAM, LdT
			D	236T, 236Y	4.59%	ADV, TDF
				250G, 250H	2.29%	ETV
Singla et al. 2015	Chandigarh	On Treatment	C	L80V M204I	2.89% 2.89%	LAM, LdT, ETV
			D	Q215S	1.44%	ADV
				P237T + N238H	1.44%	ADV
Tuteja et al. 2014	New Delhi	Naïve	A	L80	7.48%	LAM/LdT
				N236I	2.67%	ADV
				M250V	2.67%	ETV
			D	L80	3.74%	LAM, LdT
				N236I	4.27%	ADV
				M250V	4.27%	ETV
Panigrahi et al, 2013	Kolkata	On Treatment, Naïve	D	Q215	4.70%	
			C	Q215	1.17%	
Singla et al. 2013	Chandigarh	Naïve	D	S202R+ M250I,	1.40%	ETV
				I233V	2.81%	ADV
			C	M204I	2.81%	LAM, ETV, LdT
International Studies						
Ababneh et al. 2019	Amman, Jordan	On Treatment	D	L180M, M204V, V173L, T184A	8.10%	LAM, LdT, ETV
Kim et al. 2017	South Korea	Naïve	C	T184A/C/F	0.76%	ETV
				M204I/V	6.87%	LMV,ETV, TNF
				L80I	3.81%	LMV
				L180M	2.29%	LMV, ETV, LdT

Alacam et al. 2018	Istanbul, Turkey	Naïve On Treatment	–	M204I L180M M204V L80V/I S202G/C/I A181T	22.01 % 21.07 % 17.30 % 16.67 % 5.97% 7.55%	LAM ADV LAM, LdT, ETV LAM, LdT ETV ADV
Pachecoet al. 2017	Salvador, Brazil	Naïve	A	S202I A194T M204I L180M+M204V	0.52% 0.52% 0.52% 0.52%	ETV TDF LAM LAM,ETV
			F	A181S	0.52%	LAM, ADV
Galindo et al. 2015	Mexico	1 Treated 3 Naïve	H	I169M Q215E	4.54% 9.09%	ETV LAM
			G	L180, M204V	4.54%	LAM

Table2. HBV Genotypes reported in India

Scientific group	**Place**	**Genotypes**					
		A	**D**	B	C	E	F
Suhaib et al. 2019	Jaipur	**1/50 (98%)**	49/50 (2%)	-	-	-	-
Kumar and Singh 2019	Haryana	21-Feb -9.52%	**19/21 (90%)**	-	-	-	-
Khan et al. 2018	Uttar Pradesh	5/16 (31.25%)	-	-	-	-	-
Chavan et al. 2017	Pune	4/98 (4.08%)	**76/98 (77.55%)**	-	18/98 (18.37%)	-	-
Agarwal et al. 2015	Cochin	2/10 (20%)	**8/10 (80%)**	-	-	-	-
Banerjee et al.2014	Kolkata	3/76 (3.94%)	**68/76 (89.5%)**	-	May-76 -6.60%	-	-
Biswas et al. 2013	Kolkata	4/47 (8.51%)	**41/47 (87.23%)**	-	Feb-47 -4.26%	-	-
Kumar et al. 2011	Varanasi	**81/150 (54%)**	32/150 (21.3%)	10/150 (6.7%)	15/150 (10%)	9/150 (6%)	3/150 (2%)

6. Diagnosis

Serological markers for hepatitis B infection- The seroprevalence of various HBV infection markers among infants born before and after the introduction of hepatitis B immunisation in phase-1 states allowed researchers to examine the impact of the vaccine. During each of the stages of HBV infection, at least one serologic marker is present. All indicators except HBcAg have commercially available serologic assays since no free HBcAg circulates in blood (Shah et al., 2021). Serological indicators are essential for tracking therapy effectiveness and anticipating problems.Chronic HBV infection has a wide range of serologic characteristics. Hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs), hepatitis B core antigen (HBcAg) and antibody to HBcAg (anti-HBc), and hepatitis B e antigen (HBeAg) and antibody to HBeAg are antigens and antibodies associated with HBV infection (anti-HBe). HBcrAg has a better connection with antiviral therapy-induced decreases in HBV DNA levels and intrahepatic HBV cccDNA levels. It can also be used to predict HBV reactivation in immune compromised people and the onset of HCC (Shinkai et al., 2013; Kim et al., 2015; Honer et al., 2018; Mak et al., 2019). HBV RNA, a pregenomic RNA-containing virion comparable to HBcrAg is another relatively new biomarker. When compared to HBV DNA blood levels, treatment naive CHB patients exhibit lower serum levels of HBV RNA (by 1-2 logs) (Wang et al., 2016; Butler et al., 2018). HBV RNA levels are considerably greater than HBV DNA levels in patients receiving NAs, making it a predictor of response. Both HBV DNA and HBsAg titers have a strong linear connection with HBV RNA (Gao et al., 2017).

The covalently closed circular DNA (cccDNA) protein serves as a template for viral protein transcription and translation. The fundamental mechanism for infection reactivation after treatment discontinuation is the persistence of cccDNA within the nucleus of infected hepatocytes despite treatment and viral suppression (Durantel, Zoulim, 2016).Hepatitis B core-related antigen (HBcrAg) is a mixture of three related viral proteins (HBcAg, HBeAg, and a truncated 22kDa precore protein) generated from multiple viral proteins (Inoue and Tanaka, 2019). In individuals treated with NAs, both HBcrAg and HBV RNA can predict long-term off-therapy HBV virological control. Chang et al. validated HBcrAg levels to represent on-treatment hepatic fibrosis progression, and therefore its significance in monitoring hepatic histological alterations in a recent prospective trial (Chang et al., 2019). Serial liver biopsies are required for studies linking new biomarkers with hepatic fibrosis and cccDNA, resulting in smaller sample sizes. Brakenhoff et al., 2021 found that HBV RNA decline without accompanying viral antigen fall is linked to a poor likelihood of sustained response and hepatitis B surface antigen loss in a recent trial. Future trials should evaluate the kinetics of combination biomarkers to measure antiviral efficacy, according to this study.

6.1Provision of safe blood and blood products

Screening for transfusion-transmitted illnesses (TTIs) is inefficient, poorly controlled, and poorly enforced, according to an assessment of blood transfusion procedures in India. To prevent the spreading of disease from blood, blood banking practices must be thoroughly scrutinized. The use of nucleic acid testing to

prevent HBV transmission in blood donors has been considered, although this raises the expense of screening and is not generally advised. However, there is certainly a need to more precisely map out areas of high endemicity within each state, particularly tribal communities, which are known to have extremely high incidence and should be the focus of rigorous screening and protection measures (Daniel and Syria, 2018).

8. Treatment andPrevention

Acute hepatitis B does not have any specific treatment. Therefore, the treatment is focused on ensuring comfort and a healthy nutritional balance, as well as replacing fluids lost due to vomiting and diarrhoea. The most important thing is to avoid taking medications that aren't necessary. Acetaminophen, paracetamol, and anti-vomiting medications should all be avoided. Medicines, particularly oral antiviral medications, can be used to treat chronic hepatitis B infection. Cirrhosis can be slowed, liver cancer can be prevented, and long-term survival can be improved with treatment. Depending on the setting and eligibility criteria, WHO estimates that 12% to 25% of patients with chronic hepatitis B infection will require treatment by 2021. The most potent medications to inhibit the hepatitis B virus, according to the WHO, are oral therapies (tenofovir or entecavir). The majority of patients who begin hepatitis B therapy must maintain it for the rest of their lives. Most persons with liver cancer die within months of being diagnosed. Patients in developed nations are admitted to hospitals earlier in the course of their illness and have access to surgery and chemotherapy, which can extend their lives by months or years. In high-income nations, liver transplantation is occasionally done in persons with cirrhosis or liver cancer, with different degree**s** of success (Bismuth et. al. 1999)**.**

Prevention

HBV infection can be prevented by taking universal precautions in health-care settings, such as avoiding needle-stick injuries and instituting post-exposure prophylaxis. The use of blood transfusions without a clear indication should also be limited. Most high-risk groups tend to have a high prevalence of occult infection, which necessitates more frequent screening (Schillie et al., 2018). According to published guidelines, patients must receive proper counseling on transmission prevention, lifestyle advice (e.g., avoiding high-risk sex, diet, alcohol use, and other predisposing factors such as unsafe injection practises and tattooing), and the importance of adhering to long-term treatment regimens (CDC, 2020)

Vaccination

The most important step in treatment is to avoid HBV infection through immunisation. In India, a universal immunization program (including hepatitis B vaccine) was launched in 1985 and incorporated into the Child Survival and Safe Motherhood Programme in 1992. The World Health Organization advises HB vaccination at birth, followed by two or three doses thereafter (WHO, 2019). Following the introduction of HB vaccination, some nations have seen a significant decrease in the prevalence of chronic HB virus infection as well as the rate of development of hepatocellular carcinoma. (Nelson et al., 2016). According to a cost-effectiveness analysis, including the hepatitis B vaccine in India's national immunization program will reduce the HBV carrier rate from 4.0 percent to 1.15

percent. Hepatitis B vaccination was first tried in 2002-03 and then incorporated into the National Rural Health Mission in 2005. It was first implemented in 2003 in a few districts and cities, and after its success, it was adopted by ten states in 2008, with full-country coverage beginning in 2011 (Ray, 2017). Infant hepatitis B immunization is one of the most important strategies for reducing the hepatitis B epidemic. In 2002–2003, India piloted the HB vaccine in 14 cities and 33 districts as part of the Universal Immunization Program (UIP), which was later expanded to ten states in 2007–2008 (Phase-1) and the entire country in 2011–2012 (Phase-2) (Lahariya et al., 2013). Despite the fact that the majority of children receive immunization services through the government, the private sector has played a significant role in vaccine delivery in high-income areas (Sharma et al., 2016). Serological surveys assessing the prevalence of several HBV infection markers such as hepatitis B surface antigen (HBsAg), antibodies to core antigen (anti-HBc), and surface antigen (anti-HBs) are recommended to assess the impact of the Hep-B vaccination program. A few studies in India have looked at the impact of HB vaccination in a small geographic area (Aggarwal et al., 2014; Bhattacharya et al., 2015). Hepatitis B vaccine coverage in India grew from 28.9% (urban: 43.7 percent, rural: 23.2 percent) in 2007–2008 to 62.8 percent (urban: 63.3 percent, rural: 62.5 percent) in 2015–2016 (International Institute for Population Sciences (IIPS), 2010) (Government of India - Ministry of Health and Family Welfare, 2020). It's possible that the decreased proportion of children with anti-HBs in this survey is attributable to poorer hepatitis B vaccine coverage. Anti-HBs titers are known to fall over time following vaccination, so it's possible that we underestimated the true prevalence of children who were vaccinated. Only 60% to 85% of vaccinated persons had anti-HBs titers above the generally used cut-off of 10 mIU/mL 5–7 years after completing a 3-dose immunization schedule, according to follow-up studies from numerous countries (Aggarwal et al., 2014; Bhattacharya et al., 2015). Anti-HBs were discovered in roughly 14% of children from phase-2 states born before the hepatitis B vaccine was included in the national program, according to the WHO survey. This strongly shows that youngsters from the private sector were vaccinated. The prevalence of HBsAg positive was similarly lower in children from states where the vaccination was originally offered. According to the findings of Ali et al study's children in India have a low prevalence of chronic hepatitis B virus infection. However, in the northeast and northern states, the prevalence was higher. The fact that less than 40% of infants born after the introduction of the HB-vaccine had anti-HBs as a result of immunization highlights the need to enhance India's three-dose HB vaccination coverage. The study conducted by Ali et al might be considered an interim assessment of the hepatitis B vaccine's impact, indicating that India is on track to meet the South East Asia Regional goal of 1% HBsAg prevalence among 5-year-old children (WHO, 2016).

The meaningful impact on total adults takes at least 15-20 years from the time of vaccination; the timing is still not ripe to notice this impact. In the country like India, the type of HBV vaccination, the number of doses, the volume of each dose, and the period between doses (including boosters) are all crucial factors in lowering the cases (Nelson et al., 2017). Vaccination has significantly lowered the

incidence of Hepatitis B in the Western world. Because the majority of the population in rural areas is being vaccinated, the cases are expected to reduce in India as well (Khan et al., 2019).

To complete the vaccination series, WHO advises that all babies receive the hepatitis B vaccine as soon as possible after delivery, preferably within 24 hours, followed by 2 or 3 doses of hepatitis B vaccine spaced at least 4 weeks a part. The protection lasts at least 20 years and is likely to last a lifetime (NCDC, 2011). Booster immunizations are not recommended for people who have completed the 3-dose vaccination programme, according to the WHO. Antiviral prophylaxis, in addition to newborn vaccination, is recommended by WHO to prevent hepatitis B transmission from mother to child. Blood safety initiatives and safer sex habits, such as limiting the number of partners and using barrier protective measures (condoms), can also help to prevent transmission (Schillie et al., 2018).

To reduce the global burden of HBV-related HCC, efforts in the development of preventative interventions are urgently needed. GOI has launched hepatitis control program on the occasion of the World Hepatitis Day, 28th July 2018. According to Yang et al, the three layers of preventive methods, namely primary, secondary, and tertiary prevention, all these are effective in preventing HBV-related HCC (Yang et al., 2019)[Figure 2]. Prevention is important as, the incidence and mortality of HBV-related HCC have progressively grown in recent years.

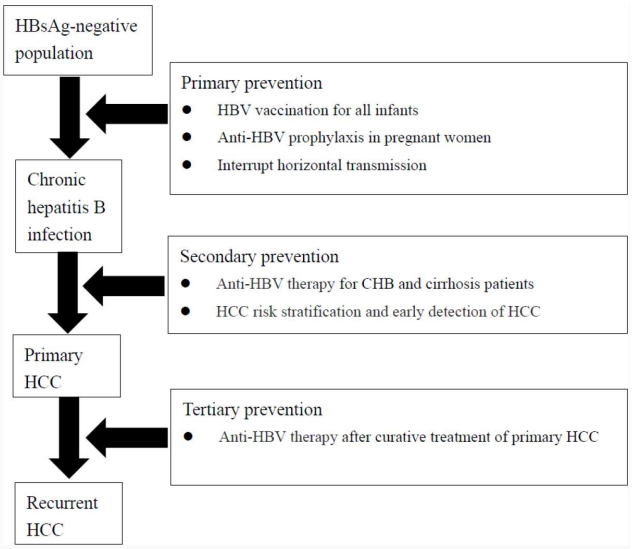

Figure 2. Preventive strategies of HBV-related HCC. HBV: hepatitis B virus; HCC: hepatocellular carcinoma. (Lin and Kao, 2021)

9. Conclusion

Hepatitis B and C kill over a million people worldwide. Hepatitis B virus (HBV) and hepatitis C virus (HCV) jointly cause the majority of viral hepatitis, which kills more people each year than diabetes, traffic accidents and HIV/AIDS combined. During the early 2000s, although deaths from other major killers (such as malaria, tuberculosis, and HIV) decreased, viral hepatitis deaths increased (IHME, 2016). These deaths could have been avoided by the vaccination as HBV vaccine has a 95% protective rate after three doses. Although HBV infection cannot be cured, it can be treated until the viral burden is undetectable (EASL, 2012). Despite the fact that there is no vaccine for HCV, emerging therapeutic medicines can cure the illness in over 95 percent of individuals (Afdhal et al., 2014; Charlton et al., 2015; Feld et al., 2014). Increased prevention and improved access to viral hepatitis therapies could drastically lower the number of infected people.

By 2030, the World Health Organization (WHO) forecasts that lowering chronic hepatitis B and C incidence by 90% and mortality by 65% will save 7.1 million lives. (WHO, 2016).

References

1. Abu-Amara, Mahmoud, Orlando Cerocchi, Gurtej Malhi, Suraj Sharma, ColinaYim, Hemant Shah, David K. Wong, Harry LA Janssen, and Jordan J. Feld. "The applicability of hepatocellular carcinoma risk prediction scores in a North American patient population with chronic hepatitis B infection." Gut 65, no. 8 (2016): 1347-1358.
2. Aggarwal, R., Babu, J. J., Hemalatha, R., Reddy, A. V., Sharma, D., & Kumar, T. (2014). Effect of inclusion of hepatitis B vaccine in childhood immunization program in India: a retrospective cohort study. Indian pediatrics, 51(11), 875-879.
3. Alfaiate, Dulce, Julie Lucifora, Natali Abeywickrama-Samarakoon, Maud Michelet, Barbara Testoni, Jean-Claude Cortay, Camille Sureau, Fabien Zoulim, Paul Dény, and David Durantel. "HDV RNA replication is associated with HBV repression and interferon-stimulated genes induction in super-infected hepatocytes." Antiviral research 136 (2016): 19-31.
4. Andernach, I. E., Hunewald, O. E., & Muller, C. P. (2013). Bayesian inference of the evolution of HBV/E. PLoS One, 8(11), e81690.
5. Araujo, N. M., Waizbort, R., & Kay, A. (2011). Hepatitis B virus infection from an evolutionary point of view: how viral, host, and environmental factors shape genotypes and subgenotypes. Infection, Genetics and Evolution, 11(6), 1199-1207.
6. Bhattacharya, H., Bhattacharya, D., Ghosal, S. R., Roy, S., &Sugunan, A. P. (2015). Status of hepatitis B infection-a decade after hepatitis B vaccination of susceptible Nicobarese, an indigenous tribe of Andaman & Nicobar (A&N) islands with high hepatitis B endemicity. The Indian journal of medical research, 141(5), 653.
7. Bismuth, H., Majno, P. E., & Adam, R. (1999). Liver transplantation for hepatocellular carcinoma. In Seminars in liver disease (Vol. 19, No. 03, pp. 311-322). © 1999 by Thieme Medical Publishers, Inc..
8. Bouckaert, R., Alvarado-Mora, M. V., &Pinho, J. R. (2013). Evolutionary rates and HBV: issues of rate estimation with Bayesian molecular methods. AntivirTher, 18(3 Pt B), 497-503.
9. Brakenhoff, S. M., de Man, R. A., de Knegt, R. J., Janssen, H. L., &Sonneveld, M. J. (2021). HBV cure—the quest for biomarkers to predict off-treatment sustained response. Authors' reply.
10. Butler, E. K., Gersch, J., McNamara, A., Luk, K. C., Holzmayer, V., de Medina, M., ... &Cloherty, G. A. (2018). Hepatitis B virus serum DNA and RNA levels in nucleos (t) ide analog-treated or untreated patients during chronic and acute infection. Hepatology, 68(6), 2106-2117.
11. Carman, W. F., Karayiannis, P., Waters, J., Thomas, H. C., Zanetti, A. R., Manzillo, G., & Zuckerman, A. T. (1990). Vaccine-induced escape mutant of hepatitis B virus. The lancet, 336(8711), 325-329.
12. Cho, E. J., Kim, S. E., Suk, K. T., An, J., Jeong, S. W., Chung, W. J., & Kim, Y. J. (2017). Current status and strategies for hepatitis B control in Korea. Clinical and molecular hepatology, 23(3), 205.
13. Chu, C. M., &Liaw, Y. F. (2006, May). Hepatitis B virus-related cirrhosis: natural history and treatment. In Seminars in liver disease (Vol. 26, No. 02, pp. 142-152).

14. Cooreman, M. P., Leroux-Roels, G., &Paulij, W. P. (2001). Vaccine-and hepatitis B immune globulin-induced escape mutations of hepatitis B virus surface antigen. Journal of biomedical science, 8(3), 237-247.

15. Datta, S., Chatterjee, S., Veer, V., & Chakravarty, R. (2012). Molecular biology of the hepatitis B virus for clinicians. Journal of clinical and experimental hepatology, 2(4), 353-365.

16. Din, A., Li, Y., & Shah, M. A. (2021). The complex dynamics of Hepatitis B infected individuals with optimal control. Journal of Systems Science and Complexity, 1-23.

17. Duong, C. M., Olszyna, D. P., &McLaws, M. L. (2015). Hepatitis B and C virus infections among patients with end stage renal disease in a low-resourced hemodialysis center in Vietnam: a cross-sectional study. BMC public health, 15(1), 1-7.

18. European Association For The Study Of The Liver. (2017). EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. Journal of hepatology, 67(2), 370-398.

19. FitzSimons, D., Francois, G., De Carli, G., Shouval, D., Prüss-Üstün, A., Puro, V., ... & Van Damme, P. (2008). Hepatitis B virus, hepatitis C virus and other blood-borne infections in healthcare workers: guidelines for prevention and management in industrialised countries. Occupational and Environmental Medicine, 65(7), 446-451.

20. Gao, Yuhua, Yutang Li, Qinghua Meng, Zhanqing Zhang, Ping Zhao, Qinghua Shang, Yue Li et al. "Serum hepatitis B virus DNA, RNA, and HBsAg: which correlated better with intrahepatic covalently closed circular DNA before and after nucleos (t) ide analogue treatment?." Journal of clinical microbiology 55, no. 10 (2017): 2972-2982.

21. Ghosh, S., Banerjee, P., Deny, P., Mondal, R. K., Nandi, M., Roychoudhury, A., ... & Datta, S. (2013). New HBV subgenotype D 9, a novel D/C recombinant, identified in patients with chronic HB e A g-negative infection in E astern I ndia. Journal of Viral Hepatitis, 20(3), 209-218.

22. Günal, Ö., Barut, Ş., Etikan, İ., Duygu, F., Tuncel, U., &Sünbül, M. (2014). Relation between serum quantitative HBsAg, ALT and HBV DNA levels in HBeAg negative chronic HBV infection. Turk J Gastroenterol, 25(1), 142-6.

23. HönerzuSiederdissen, Christoph, Aric Josun Hui, Wattana Sukeepaisarnjaroen, PisitTangkijvanich, Wei Wen Su, Gerardo Enrique Guillén Nieto, Paul Gineste et al. "Contrasting Timing of Virological Relapse After Discontinuation of Tenofovir or Entecavir in Hepatitis B e Antigen–Negative Patients." The Journal of infectious diseases 218, no. 9 (2018): 1480-1484.

24. Ibrahim, N., & Idris, A. (2014). Hepatitis B awareness among medical students and their vaccination status at Syrian Private University. Hepatitis research and treatment, 2014.

25. Iida-Ueno, A., Enomoto, M., Tamori, A., & Kawada, N. (2017). Hepatitis B virus infection and alcohol consumption. World journal of gastroenterology, 23(15), 2651.

26. Inoue, Jun, Masashi Ninomiya, TeruyukiUmetsu, Takuya Nakamura, Takayuki Kogure, Eiji Kakazu, Tomoaki Iwata et al. "Small interfering RNA screening for the small GTPase Rab proteins identifies Rab5B as a major regulator of hepatitis B virus production."

Journal of virology 93, no. 15 (2019): e00621-19.
27. Kay, A., &Zoulim, F. (2007). Hepatitis B virus genetic variability and evolution. Virus research, 127(2), 164-176.
28. Khalili, M., Shuhart, M. C., Lombardero, M., Feld, J. J., Kleiner, D. E., Chung, R. T, Lok, A. S. (2018). Relationship between metabolic syndrome, alanine aminotransferase levels, and liver disease severity in a multiethnic north American cohort with chronic hepatitis B. Diabetes care, 41(6), 1251-1259.
29. Khan, J., Shil, A., & Mohanty, S. K. (2019). Hepatitis B vaccination coverage across India: exploring the spatial heterogeneity and contextual determinants. BMC Public Health, 19(1), 1-14.
30. Kidd-Ljunggren, K., Miyakawa, Y., & Kidd, A. H. (2002). Genetic variability in hepatitis B viruses. Journal of General Virology, 83(6), 1267-1280.
31. Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. Intervirology, 57(3-4), 141-150.
32. Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. Intervirology, 57(3-4), 141-150.
Komatsu H, Inui A, and Fujisawa T, "The role of body fluidsin the horizontal transmission of hepatitis B virus via household/close contact," EMJ, vol. 1, no. 1, pp. 68–75, 2016.
33. Lahariya, C., Subramanya, B. P., &Sosler, S. (2013). An assessment of hepatitis B vaccine introduction in India: Lessons for roll out and scale up of new vaccines in immunization programs. Indian journal of public health, 57(1), 8.
34. Lahariya, C., Subramanya, B. P., &Sosler, S. (2013). An assessment of hepatitis B vaccine introduction in India: Lessons for roll out and scale up of new vaccines in immunization programs. Indian journal of public health, 57(1), 8.
35. Lazarevic, I., Banko, A., Miljanovic, D., &Cupic, M. (2019). Immune-escape hepatitis B virus mutations associated with viral reactivation upon immunosuppression. Viruses, 11(9), 778.
36. Lin, C. L., & Kao, J. H. (2011). The clinical implications of hepatitis B virus genotype: Recent advances. Journal of gastroenterology and hepatology, 26, 123-130.
37. Lin, C. W., Lin, C. C., Mo, L. R., Chang, C. Y., Perng, D. S., Hsu, C. C., ... & Yang, S. S. (2013). Heavy alcohol consumption increases the incidence of hepatocellular carcinoma in hepatitis B virus-related cirrhosis. Journal of hepatology, 58(4), 730-735.
38. Liu, C. J., & Kao, J. H. (2013, May). Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. In Seminars in liver disease (Vol. 33, No. 02, pp. 097-102). Thieme Medical Publishers.
39. Locarnini, S., & Mason, W. S. (2006). Cellular and virological mechanisms of HBV drug resistance. Journal of hepatology, 44(2), 422-431.
40. Lucifora, J., Arzberger, S., Durantel, D., Belloni, L., Strubin, M., Levrero, M. Protzer, U. (2011). Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. Journal of hepatology, 55(5), 996-1003.
41. Mak, L. Y., Seto, W. K., Fung, J., & Yuen, M. F. (2019). New biomarkers of chronic hepatitis B. Gut and liver, 13(6), 589.
42. Mogul, D. B., Brereton, N., Carson, K. A., Pittarelli, M., Daniel, H., Torbenson, M., & Schwarz, K. B. (2018). Development of a Dietary Methyl Donor Food Frequency

Questionnaire to Assess Folate and Vitamin B12 Status in Children with Chronic Hepatitis B Virus Infection. The Journal of pediatrics, 203, 41-46.

43. Moorman AC, de Perio MA, Goldschmidt R, Chu C, Kuhar D, Henderson DK, Naggie S, Kamili S, Spradling PR, Gordon SC, Russi MB. Testing and Clinical Management of Health Care Personnel Potentially Exposed to Hepatitis C Virus-CDC Guidance, United States, 2020. MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports. 2020 Jul 24;69(6):1-8.

44. Moura, I. F., Lopes, E. P., Alvarado-Mora, M. V., Pinho, J. R., &Carrilho, F. J. (2013). Phylogenetic analysis and subgenotypic distribution of the hepatitis B virus in Recife, Brazil. Infection, Genetics and Evolution, 14, 195-199.

45. Nelson, N. P., Easterbrook, P. J., & McMahon, B. J. (2016). Epidemiology of hepatitis B virus infection and impact of vaccination on disease. Clinics in liver disease, 20(4), 607-628.

46. Ntziora, F., D. Paraskevis, C. Haida, E. Magiorkinis, E. Manesis, G. Papatheodoridis, S. Manolakopoulos et al. "Quantitative detection of the M204V hepatitis B virus minor variants by amplification refractory mutation system real-time PCR combined with molecular beacon technology." Journal of clinical microbiology 47, no. 8 (2009): 2544-2550.

47. Pan, Calvin Q., Zhongping Duan, Erhei Dai, Shuqin Zhang, Guorong Han, Yuming Wang, Huaihong Zhang et al. "Tenofovir to prevent hepatitis B transmission in mothers with high viral load." New England journal of medicine 374, no. 24 (2016): 2324-2334.

48. Paraskevis, D., Magiorkinis, G., Magiorkinis, E., Ho, S. Y., Belshaw, R., Allain, J. P., &Hatzakis, A. (2013). Dating the origin and dispersal of hepatitis B virus infection in humans and primates. Hepatology, 57(3), 908-916.

49. Paraskevis, D., Magiorkinis, G., Magiorkinis, E., Ho, S. Y., Belshaw, R., Allain, J. P., &Hatzakis, A. (2013). Dating the origin and dispersal of hepatitis B virus infection in humans and primates. Hepatology, 57(3), 908-916.

50. Pastor, Rebecca, François Habersetzer, Samira Fafi-Kremer, Michel Doffoël, Thomas F. Baumert, Jean-Pierre Gut, Françoise Stoll-Keller, and Evelyne Schvoerer. "Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naive patients." World Journal of Gastroenterology: WJG 15, no. 6 (2009): 753.

51. Pathoumthong, K., Khampanisong, P., Quet, F., Latthaphasavang, V., Souvong, V., & Buisson, Y. (2014). Vaccination status, knowledge and awareness towards hepatitis B among students of health professions in Vientiane, Lao PDR. Vaccine, 32(39), 4993-4999.

52. Patwardhan, V. R., Sengupta, N., Bonder, A., Lau, D., &Afdhal, N. H. (2014). Treatment cessation in noncirrhotic, e-antigen negative chronic hepatitis B is safe and effective following prolonged anti-viral suppression with nucleosides/nucleotides. Alimentary pharmacology & therapeutics, 40(7), 804-810.

53. Perz, J. F., Armstrong, G. L., Farrington, L. A., Hutin, Y. J., & Bell, B. P. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. Journal of hepatology, 45(4), 529-538.

54. Rajoriya, N., Combet, C., Zoulim, F., & Janssen, H. L. (2017). How viral genetic variants

and genotypes influence disease and treatment outcome of chronic hepatitis B. Time for an individualisedapproach?. Journal of hepatology, 67(6), 1281-1297.

55. Revill, P. A., Tu, T., Netter, H. J., Yuen, L. K., Locarnini, S. A., & Littlejohn, M. (2020). The evolution and clinical impact of hepatitis B virus genome diversity. Nature Reviews Gastroenterology & Hepatology, 17(10), 618-634.

56. Revill, Peter A., Francis V. Chisari, Joan M. Block, Maura Dandri, Adam J. Gehring, Haitao Guo, Jianming Hu et al. "A global scientific strategy to cure hepatitis B." The Lancet Gastroenterology & Hepatology 4, no. 7 (2019): 545-558.

57. Rivero-Juárez, A., Camacho, A., Merchante, N., Pérez-Camacho, I., Macias, J., Ortiz-Garcia, C., ... & Rivero, A. (2013). Incidence of liver damage of uncertain origin in HIV patients not co-infected with HCV/HBV. PLoS One, 8(7), e68953.

58. Rivero-Juárez, A., Camacho, A., Merchante, N., Pérez-Camacho, I., Macias, J., Ortiz-Garcia, C., ... & Rivero, A. (2013). Incidence of liver damage of uncertain origin in HIV patients not co-infected with HCV/HBV. PLoS One, 8(7), e68953.

59. Sakamoto, T., Tanaka, Y., Watanabe, T., Iijima, S., Kani, S., Sugiyama, M., ... &Mizokami, M. (2013). Mechanism of the dependence of hepatitis B virus genotype G on co-infection with other genotypes for viral replication. Journal of viral hepatitis, 20(4), e27-e36.

60. Sarin, S. K., Kumar, M., Lau, G. K., Abbas, Z., Chan, H. L. Y., Chen, C. J., Kao, J. H. (2016). Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatology international, 10(1), 1-98.

61. Schillie, S., Harris, A., Link-Gelles, R., Romero, J., Ward, J., & Nelson, N. (2018). Recommendations of the Advisory Committee on Immunization Practices for use of a hepatitis B vaccine with a novel adjuvant. Morbidity and Mortality Weekly Report, 67(15), 455.

62. Schillie, S., Harris, A., Link-Gelles, R., Romero, J., Ward, J., & Nelson, N. (2018). Recommendations of the Advisory Committee on Immunization Practices for use of a hepatitis B vaccine with a novel adjuvant. Morbidity and Mortality Weekly Report, 67(15), 455.

63. Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G., & Ott, J. J. (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet, 386(10003), 1546-1555.

64. Seeger, C., & Mason, W. S. (2000). Hepatitis B virus biology. Microbiology and molecular biology reviews, 64(1), 51-68.

65. Seeger, C., & Mason, W. S. (2000). Hepatitis B virus biology. Microbiology and molecular biology reviews, 64(1), 51-68.

66. Seeger, C., & Mason, W. S. (2013). Sodium-dependent taurocholic cotransporting polypeptide: a candidate receptor for human hepatitis B virus. Gut, 62(8), 1093-1095.

67. Shin, A., Cho, E. R., Kim, J., Sung, J., Park, K. W., Lim, M. K., & Shin, H. R. (2009). Factors associated with awareness of infection status among chronic hepatitis B and C carriers in Korea. Cancer Epidemiology and Prevention Biomarkers, 18(6), 1894-1898.

68. Shinkai, Noboru, Kentaro Matsuura, FuminakaSugauchi, Tsunamasa Watanabe, Shuko Murakami, Etsuko Iio, Shintaro Ogawa, ShunsukeNojiri, Takashi Joh, and Yasuhito Tanaka. "Application of a newly developed high-sensitivity HBsAg chemiluminescent enzyme

immunoassay for hepatitis B patients with HBsAg seroclearance." Journal of clinical microbiology 51, no. 11 (2013): 3484-3491.

69. Solmone, M., Vincenti, D., Prosperi, M. C. F., Bruselles, A., Ippolito, G., &Capobianchi, M. R. (2009). Use of massively parallel ultradeep pyrosequencing to characterize the genetic diversity of hepatitis B virus in drug-resistant and drug-naive patients and to detect minor variants in reverse transcriptase and hepatitis BS antigen. Journal of virology, 83(4), 1718-1726.

70. Song, L. W., Liu, P. G., Liu, C. J., Zhang, T. Y., Cheng, X. D., Wu, H. L., ... & Xia, N. S. (2015). Quantitative hepatitis B core antibody levels in the natural history of hepatitis B virus infection. Clinical Microbiology and Infection, 21(2), 197-203.

71. Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., ... &Mizokami, M. (2009). A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. Journal of virology, 83(20), 10538-10547.

72. Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., ... &Mizokami, M. (2009). A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. Journal of virology, 83(20), 10538-10547.

73. Thornton AC, Jose S, Bhagani S, Chadwick D, Dunn D, Gilson R, Main J, Nelson M, Rodger A, Taylor C, Youssef E. Hepatitis B, hepatitis C, and mortality among HIV-positive individuals. AIDS (London, England). 2017 Nov 28;31(18):2525.

74. Tozun, N., O. Ozdogan, Y. Cakaloglu, R. Idilman, Z. Karasu, U. L. U. S. Akarca, S. Kaymakoglu, and O. Ergonul. "Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study." Clinical Microbiology and Infection 21, no. 11 (2015): 1020-1026.

WHO, World Hepatitis Day 2018, World Health Organization, 2018, https://www.who.int/campaigns/world-hepatitisday/2018.

75. Yeo, Yee Hui, Hsiu J. Ho, Hwai-I. Yang, Tai-Chung Tseng, Tetsuya Hosaka, Huy N. Trinh, Min-Sun Kwak et al. "Factors associated with rates of HBsAg seroclearance in adults with chronic HBV infection: a systematic review and meta-analysis." Gastroenterology 156, no. 3 (2019): 635-646.

76. Yu, H., Yuan, Q., Ge, S. X., Wang, H. Y., Zhang, Y. L., Chen, Q. R., ... & Xia, N. S. (2010). Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype “I”. PloS one, 5(2), e9297.

77. Yuan, J. M., Ambinder, A., Fan, Y., Gao, Y. T., Mimi, C. Y., &Groopman, J. D. (2009). Prospective evaluation of hepatitis B 1762T/1764A mutations on hepatocellular carcinoma
development in Shanghai, China. Cancer Epidemiology and Prevention Biomarkers, 18(2), 590-594.

78. Zhang, X., Zhang, H., & Ye, L. (2006). Effects of hepatitis B virus X protein on the development of liver cancer. Journal of Laboratory and Clinical Medicine, 147(2), 58-66.

79. Zuckerman, J. N., & Zuckerman, A. J. (2003). Mutations of the surface protein of hepatitis B virus. Antiviral research, 60(2), 75-78.

Y.-F. Liaw and C.-M. Chu, “Hepatitis B virus infection,” TheLancet, vol. 373, no. 9663, pp. 582–592, 2009