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Review

Hepatitis B Virus (HBV) Genotypes, Serotypes, and Laboratory Detections: A Comprehensive Review

Essa M. Atafi[1*], Naser A. Alkenani[1], Ishtiaq Qadri[1], Bassam O. Al johny[1], Waleed Y. Shami[2], Moussa D. Moharag[2], Khaled A. Ghazwani[3]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Saudi Arabia. P.O. Box 80203 Jeddah 21589.

[2]Bish General Hospital, Jazan Health Cluster, Saudi Arabia. P.O. Box 87743, Jazan.

[3]Central Blood Bank, Jazan General Hospital, jazan, Saudi Arabia.

[*]**Corresponding Author's E-mail: eahmedatafi@stu.kau.edu.sa Topeissa@hotmail.com**

Abstract

Hepatitis B virus (HBV) exhibits significant genetic and antigenic variability, influencing its clinical manifestations, diagnostic approaches, and response to treatment. This review comprehensively overviews HBV genotypes, serotypes, and laboratory detection techniques. The diversity of HBV genotypes, their geographic distribution, and the clinical implications of specific genotypes and serotypes are explored. The article also highlights advances in laboratory detection methods, including molecular diagnostics and serological assays, essential for effective disease management and monitoring. The current study utilized a systematic review approach to identify relevant articles from databases including PubMed, MDPI, Scopus, and Research Gate, applying specific search criteria. A deeper understanding of HBV's genetic and serological diversity is critical for developing targeted therapies and improving global public health outcomes.

Keywords: Genotypes, Hepatitis B Virus, Laboratory Detections, Serotypes

INTRODUCTION

Hepatitis B virus (HBV) is a partially double-stranded DNA virus belonging to the *Hepadnaviridae* family [1, 2]. Globally, HBV remains a significant public health challenge, affecting over 2 billion individuals and causing chronic infections in approximately 257 million people [3]. The virus is primarily transmitted through parenteral, sexual, perinatal, and horizontal routes, and it is a leading cause of liver-related morbidity and mortality, including cirrhosis, hepatocellular carcinoma (HCC), and liver failure [4,5]. HBV infection outcomes vary widely among individuals due to multiple factors, including viral genetic diversity, host immune responses, and environmental influences [6]. The viral genome exhibits high genetic variability, resulting in the classification of HBV into nine recognized genotypes (A to I) and a proposed tenth genotype (J) based on >8% sequence divergence [7]. These genotypes display distinct geographic distributions and are associated with variable clinical presentations, disease progression, and responses to antiviral therapy. Certain genotypes, such as genotype C prevalent in East Asia, are linked to a higher risk of chronic hepatitis, cirrhosis, and HCC. In contrast genotype B, also found in East Asia, is associated with a milder disease course [8,9]. Similarly, genotype A, common in Europe, North America, and sub-Saharan Africa, typically correlates with less severe outcomes [10]. In Saudi Arabia, genotype D has been implicated in more severe liver disease and higher HCC risk compared to other genotypes, underscoring the need for region-specific studies to guide diagnosis, treatment, and prevention strategies [11]. In addition to genotypes, HBV is classified into serotypes based on antigenic variations in its surface proteins. These serotypes, such as *ayw* and *adw*, have further subtypes and associated with differing disease severities and geographic distributions [12]. While the correlation between serotypes and clinical outcomes is

less defined than for genotypes, their study provides valuable insights into HBV pathogenesis and potential therapeutic targets [13].

METHODS

Literature Search and Selection Criteria

To conduct a comprehensive review of HBV genotypes, serotypes, and laboratory detection methods, a systematic approach was followed. The research questions and objectives were defined to focus on summarizing HBV genotypes' global distribution, serotypes, and diagnostic techniques. The relevant studies were retrieved using databases such as PubMed, Scopus, EMBASE, Web of Science, and WHO reports. A structured search strategy was implemented, combining keywords like “HBV,” “genotypes,” and “laboratory detection” with Boolean operators to ensure comprehensive results. Inclusion criteria targeted studies discussing HBV genotypes, serotypes, and diagnostic methods, while unrelated or low-quality studies were excluded.

Study selection was conducted through a two-step process: initial title/abstract screening followed by a full-text review using tools like Rayyan or Covidence. Data extraction captured geographic distribution, the clinical significance of genotypes and serotypes, and diagnostic techniques' sensitivity, specificity, and applications. Quality assessment was performed using tools such as the Newcastle-Ottawa Scale and QUADAS-2 to ensure the reliability of the included studies.

Data synthesis combined qualitative summaries of findings and, where applicable, quantitative meta-analysis using statistical tools like R or STATA. The review followed PRISMA guidelines, presenting study selection via a flow diagram and organizing results into thematic sections with visual representations like maps and comparative tables. Reference management tools like EndNote were used to streamline the process.

DISCUSSION

HBV Genotypes and Sub-genotypes

HBV exhibits significant genetic diversity, which has led to the identification of various HBV genotypes and sub-genotypes[14]. These variations significantly influence the virus's clinical outcomes, pathogenesis, and response to antiviral treatments [15]. The variability is largely due to the unique replication mechanism of HBV, which lacks proofreading activity in its polymerase enzyme and uses an RNA intermediate in its replication cycle. Consequently, this allows for a high rate of mutation, although not as rapid as RNA viruses due to the DNA nature of the HBV genome [15]. The overlapping open reading frames of the HBV genome further complicate the fixation of mutations, ensuring that HBV has an intermediate mutation rate between RNA and DNA viruses [16].

HBV is classified into 10 main genotypes (A to J), based on sequence variations within its genome [14, 17]. Each genotype is geographically distributed, with distinct clinical implications regarding disease progression and response to therapy.

Genotype A: Found predominantly in Europe, North America, and parts of Africa, genotype A is associated with both acute and chronic HBV infections, and in some cases, can lead to hepatocellular carcinoma (HCC). It is further subdivided into seven (A1-A7) sub-genotypes [18, 19].

Genotype B: Predominantly found in Asia, especially in China, Taiwan, and Southeast Asia, genotype B is associated with a higher risk of vertical transmission (from mother to child). However, it has a relatively better prognosis compared to other genotypes, with lower risks of progressing to chronic hepatitis, cirrhosis, or HCC [20].

Genotype C: Common in East Asia, particularly China, Japan, and Korea, genotype C is known to be linked to more severe liver disease outcomes, including chronic infection, cirrhosis, and HCC. It is further divided into sub-genotypes C1 to C6 [18, 20]. Genotype D: This genotype is prevalent in the Mediterranean region, the Middle East, Central Asia, and parts of South Asia, where it is associated with a high prevalence of chronic HBV infection and a high risk of HCC. Genotype D has sub-genotypes D1 to D8 [19, 21].

Genotype E: Predominantly found in West Africa, genotype E is often linked to chronic infection [19]. Genotype F: Found in Central and South America, genotype F has sub-genotypes F1 to F4. Similar to genotype E, genotype F is strongly associated with chronic hepatitis B [19, 21].

Genotype G: A rare genotype found in Europe, North America, and Central Asia, genotype G is associated with chronic HBV infections and may contribute to the development of cirrhosis and HCC [18, 19].

Genotypes H, I, and J: Genotype H is predominantly found in Central America, genotype I in Vietnam, and genotype J in Central Asia. Genotypes H and I are associated with chronic hepatitis and liver disease progression, while the clinical significance of genotype J is still under investigation [18, 19, 22]. Genotypic variations in HBV significantly influence clinical outcomes such as hepatitis e antigen (HBeAg) seroconversion rates, viral replication, mutation patterns, and the response to antiviral therapy. In particular, certain genotypes, such as B and C, show a tendency to result in more severe disease outcomes compared to others [19, 23].

Evolutionary Patterns of HBV Genotypes

The evolution of HBV genotypes is shaped by multiple factors, including geographic distribution, host migration, transmission routes, and viral adaptation [24]. Phylogenetic studies have revealed that HBV genotypes exhibit genetic divergence, indicating independent evolutionary paths [25]. Selection pressures, such as host immune responses, antiviral treatments, and host population dynamics, contribute to the emergence of specific viral [25].

Geographic diversification and migration have led to distinct distributions of HBV genotypes, with the possibility of multiple genotypes coexisting in areas of admixture. This coexistence promotes genetic exchange and recombination between genotypes, resulting in novel recombinant strains [26]. Recombination events, particularly between genotypes and sub-genotypes, have led to the development of new HBV strains with unique genetic characteristics and clinical behaviors [27].

HBV Mutations and Their Clinical Implications

Mutations within the HBV genome significantly affect the virus's replication, immune evasion, antiviral resistance, and disease progression [28]. Point mutations, deletions, insertions, and recombination events contribute to the genetic diversity of HBV. Specific mutations within the pre-core and core regions of the HBV genome are linked to HBeAg-negative variants, which are common in genotype D [29]. Additionally, mutations in the core promoter region (e.g., A1762T and G1764A) are associated with increased viral replication and disease progression [30]. Certain mutations in the surface gene (S-gene), such as G145R in genotypes A, D, and F, and Q129H in genotype B, can lead to immune escape, making vaccination less effective [31]. Similarly, mutations in the HBV polymerase gene (P-gene) are responsible for resistance to antiviral drugs, with specific mutations like rtM204V/I associated with lamivudine resistance and rtA181T linked to adefovir resistance in genotype D [32].

HBV Splice Variants

HBV also undergoes alternative splicing, producing various splice variants that may affect viral replication, immune responses, and disease progression [33]. These splice variants include HBxSp1, HBx-delta127, HBx-L, and various HBsAg variants. Each of these variants may have altered functional properties compared to the canonical HBV proteins, such as a longer or truncated form of the HBx protein, or altered antigenicity of HBsAg [34, 35]. These splice variants can influence the viral lifecycle and contribute to immune evasion, complicating the management and treatment of HBV infections.

HBV Quasi species Variants

HBV quasispecies refer to closely related viral variants that coexist within a single host. This genetic diversity arises from the high replication rate of the virus and the error-prone nature of the HBV reverse transcriptase enzyme [36]. These variants can include point mutations, insertions, deletions, and recombination events, all of which contribute to the virus's ability to adapt and evolve under selective pressures, such as antiviral treatment or immune responses [40]. The presence of multiple quasispecies can lead to challenges in diagnosis and treatment, as standard viral load measurements may not capture the full diversity of the viral population, potentially resulting in false negatives or underestimation of viral load [36].

The dynamic nature of HBV quasispecies is implicated in the development of antiviral resistance, immune escape, and disease progression, including cirrhosis and HCC. In some cases, the emergence of drug-resistant variants or variants with altered antigenicity can undermine the efficacy of both diagnostic tests and antiviral therapies, making it crucial to monitor and analyze quasispecies over time [36].

HBV Serotypes

HBV is also classified into two major serotypes based on antigenic variations in the surface antigen (HBsAg): the adw and ayw serotypes. These serotypes are determined by the presence of two pairs of mutually exclusive serotype determinants, d/y and w/r, along with the main antigenic determinant "a" [37]. The serotypes show geographical distribution, with the ayw serotype predominantly found in Asia and the adw serotype more common in Europe and the Americas [38]. Additionally, subtypes based on amino acid sequence variation within the HBsAg protein have further expanded the classification to include several subtypes, such as adw2, adw4, and ayw1-7 [38].

The genetic diversity of HBV serotypes and subtypes has significant implications for diagnostic assays and antiviral treatments. Some diagnostic tests may show differential sensitivity based on the serotype or subtype of HBV present in the patient, which can impact both the detection of the virus and the effectiveness of vaccines and antiviral therapies [39]. Molecular and phylogenetic analysis of HBV strains, particularly complete viral genome sequencing, has increasingly replaced traditional serotyping methods, allowing for more accurate classification and a better understanding of the virus's genetic diversity [38].

Clinical Implications of HBV Variability

The genetic variability of HBV, encompassing genotypes, sub-genotypes, and quasispecies, plays a key role in determining the clinical course of infection. Different genotypes and sub-genotypes are associated with varying rates of disease progression, with some genotypes linked to more severe outcomes such as cirrhosis and HCC [40]. The ability of HBV to adapt through quasi species variants also contributes to challenges in treating chronic HBV infections, as these variants may evade immune responses or resist antiviral drugs. Furthermore, the presence of multiple genotypes or recombinant strains within a host can complicate treatment and diagnosis, underscoring the need for precise genetic characterization to inform clinical decision-making [40].

Laboratory Detections of HBV Infection

The laboratory diagnosis of HBV infection relies on blood tests that identify viral antigens, antibodies, and HBV DNA, aiding in early detection and management [41]. Screening is crucial for high-risk individuals, including those who inject drugs, engage in unprotected sex, or are born to HBV-infected mothers [42]. The choice of diagnostic method depends on the patient's symptoms, medical history, and risk factors. For symptomatic and asymptomatic patients, different tests are recommended, ranging from serological markers to advanced molecular assays [42].

Serological Diagnosis

Serological testing uses blood serum to detect HBV-related antibodies and antigens, such as HBsAg, HBeAg, and anti-HBc [42]. This method distinguishes between acute and chronic infections, monitors disease progression, and evaluates treatment response. It also screens blood donors and assesses vaccination needs for at-risk populations [43].

HBsAg and Anti-HBs

Detecting HBsAg in serum signifies active infection, while anti-HBs indicate immunity, typically from vaccination or recovery [44]. HBsAg-positive individuals are considered infectious, necessitating further tests to evaluate the infection's severity. Conversely, anti-HBs positivity confirms immunity, and its levels help assess vaccine effectiveness [42, 43]. A negative result for both markers suggests susceptibility to HBV, prompting vaccination. The dual detection of HBsAg and anti-HBs in unvaccinated individuals implies chronic HBV infection [43].

These markers play a central role in HBV screening, vaccination monitoring, and clinical management [41, 43].

HBcAg and Anti-HBc

HBcAg, a core viral protein, is detectable during acute infections but rarely during chronic phases [44]. Anti-HBc, targeting HBcAg, is present in both acute and chronic infections and serves as an indicator of past or ongoing exposure to HBV [45]. Anti-HBc remains detectable even when HBsAg is absent, making it a valuable marker in blood donor screening and occult infection diagnosis [46, 47]. Detection of anti-HBc alone may signify resolved or occult HBV infection, associated with increased risks of liver disease and hepatocellular carcinoma (HCC) [46-48].

HBeAg and Anti-HBe

HBeAg indicates active viral replication, a higher transmission risk, and progression to severe liver diseases like cirrhosis and HCC [49]. In contrast, anti-HBe suggests an immune response with reduced replication and a lower transmission risk, indicating a better prognosis [50]. These markers are essential for assessing disease progression, treatment response, and transmission risk [51]. Their presence helps distinguish between immune-tolerant and immune-clearance phases of HBV infection, guiding clinical decisions [39, 50, 51].

HBV Viral Load Assays

HBV viral load assays quantify viral DNA in the blood, offering insights into disease activity, progression risk, and treatment response [52]. Common techniques include real-time polymerase chain reaction (PCR), transcription-mediated amplification (TMA), branched DNA (bDNA) assays, and hybrid capture assays [53]. Real-time PCR, known for its high sensitivity and specificity, is the preferred method in clinical settings [54]. These assays are critical for monitoring chronic HBV infections, evaluating the need for antiviral therapy, and predicting risks of disease progression and HCC [41, 55].

Quantitative Serum HBsAg and HBeAg Assays

Quantitative assays for HBsAg (Hepatitis B surface antigen) and HBeAg (Hepatitis B e antigen) are essential for diagnosing and managing HBV infection [25]. These assays measure antigen levels in a patient's serum using techniques like chemiluminescent immunoassay (CLIA) or ELISA [56, 57]. HBsAg assays quantify antigen levels

in international units per milliliter (IU/mL). High HBsAg levels indicate active viral replication and increased risk of disease progression [56]. Monitoring these levels helps evaluate disease activity and treatment efficacy. Similarly, HBeAg assays report levels as signal-to-cutoff ratios or IU/mL, with higher levels signifying active viral replication and greater disease progression risks. These assays are crucial for determining HBV infection phases and predicting treatment response [25, 57].For patients undergoing antiviral therapy, reductions in HBsAg and HBeAg levels correlate with positive treatment responses and reduced disease progression risks. Thus, these assays provide valuable information for clinical decision-making, improving patient outcomes. Their routine application enhances understanding of disease activity and supports tailored therapeutic strategies [25, 56, 57].

Molecular Methods for HBV Detection

Molecular detection methods are indispensable for diagnosing, monitoring, and researching HBV infections [58]. They detect and quantify viral genetic material (HBV DNA or RNA) with high sensitivity and specificity [59]. Techniques include polymerase chain reaction (PCR) for DNA amplification, reverse transcription PCR (RT-PCR) for RNA detection, nucleic acid hybridization, and next-generation sequencing (NGS) [60]. These methods provide insights into HBV viral load, mutations, and genetic diversity, which are crucial for clinical management and public health strategies [60].

HBV Genotyping

Genotyping identifies HBV genotypes or strains in patients' blood, critical for understanding disease progression and treatment responses [61]. There are ten globally recognized HBV genotypes (A-J), each associated with different clinical outcomes and geographical distributions [62]. Certain genotypes are linked to increased risks of severe liver disease, such as hepatocellular carcinoma (HCC) [63, 61].

PCR-based techniques are commonly employed for genotyping, offering precise identification by comparing viral DNA sequences to references [62]. While not routinely used for all patients, genotyping is particularly valuable in cases of antiviral therapy resistance or when distinguishing acute from chronic infection [62-61]. The information obtained aids in clinical decision-making, optimizing treatment approaches and improving patient outcomes [61-63].

Viral Mutation Analysis

This technique identifies mutations in HBV's genetic material, which can arise spontaneously during replication or under selective pressures from immune responses or antiviral therapies [64]. These mutations may result in drug resistance, increased virulence, or diagnostic challenges [65].

Viral mutation analysis involves sequencing the viral genome using PCR or NGS. PCR targets specific genome regions, while NGS provides comprehensive sequencing of the entire genome [66]. By comparing sequence data to reference genomes, researchers identify mutations affecting disease progression and treatment response [67, 68]. For instance, mutations in the HBsAg gene may reduce assay sensitivity, leading to false negatives, while Pol gene mutations can confer resistance to antiviral drugs like nucleoside analogs [67, 69-71]. However, for all patients, mutation analysis is critical in cases of poor therapy response or when distinguishing viral strains. This method provides essential insights into HBV genetic diversity and evolution, guiding treatment decisions and improving outcomes [67, 69-71].

DNA Sequencing: Sanger Sequencing and NGS

DNA sequencing techniques are vital for analyzing HBV's genetic composition, aiding in diagnostics and therapy development. Two key methods are Sanger sequencing and next-generation sequencing (NGS) [68, 72-74].

Sanger Sequencing

Developed in the 1970s, this method involves chain-terminating dideoxynucleotides that halt DNA synthesis at specific points [75]. The resulting DNA fragments are analyzed via gel electrophoresis, with fluorescent dyes indicating the sequence [76]. Sanger sequencing is reliable for small DNA regions (up to 1,000 base pairs) and is widely used in genetic testing and molecular diagnostics [77, 78]. However, it is time-consuming and costly compared to modern methods like NGS. Despite limitations, its high accuracy makes it valuable for targeted sequencing applications [77-79].

Next-Generation Sequencing (NGS)

NGS is a high-throughput, cost-effective method that sequences large DNA regions or entire genomes [80]. It generates millions of short DNA reads in parallel, allowing for rapid and efficient sequencing [71]. NGS has transformed genomics, offering unparalleled insights into viral genetic diversity [81]. The vast data generated necessitate advanced bioinformatics tools for alignment, variant calling, and interpretation [66, 80, 81].

NGS has been instrumental in identifying HBV

mutations associated with drug resistance, aiding the development of new antiviral therapies [66, 80]. It has facilitated the classification of HBV genotypes, improved diagnostic precision, and identified genetic markers linked to HCC risk [66, 80]. Additionally, NGS enables precise viral load quantification, crucial for monitoring therapy efficacy [80]. By uncovering novel viral strains, NGS enhances diagnostic capabilities and informs treatment strategies. Its integration with bioinformatics has significantly advanced HBV research, diagnostics, and therapy development [66, 80, 81].

Bioinformatics analysis of NGS data

The bioinformatics analysis of NGS data encompasses several essential steps, including quality control, preprocessing and aligning reads, calling variants, annotating genetic variations, interpreting the data, integrating with other datasets, and visualizing the results [82]. This analysis necessitates the use of specialized computational tools and a strong understanding of bioinformatics, programming, and statistical methods [83]. By conducting this analysis, researchers can identify genetic variations, comprehend gene expression patterns, and investigate biological processes [82, 83]. The applications of this analysis span across genomics research, personalized medicine, and the advancement of diagnostic tools and therapeutic approaches [82, 83].

CONCLUSION

This comprehensive review highlights the global diversity of HBV genotypes and serotypes, their clinical significance, and the advancements in laboratory detection methods. The systematic approach employed in this review enabled the identification of relevant studies from various databases, ensuring the inclusion of high-quality and comprehensive data. HBV genotypes and serotypes demonstrate distinct geographic distributions and associations with disease progression, treatment response, and vaccine efficacy, underscoring their importance in clinical practice and public health strategies.

Laboratory detection methods have evolved significantly, ranging from serological assays to advanced molecular techniques like polymerase chain reaction (PCR) and next-generation sequencing. These methods provide high sensitivity and specificity, aiding in accurate diagnosis, monitoring, and epidemiological surveillance. However, challenges remain, including the need for standardized protocols, accessibility to advanced technologies in resource-limited settings, and addressing diagnostic gaps in mixed or atypical HBV infections.

This review underscores the importance of integrating genotype and serotype data with laboratory diagnostics to improve HBV management and control. Future research should focus on refining diagnostic tools, exploring the clinical implications of emerging HBV variants, and strengthening global efforts to eradicate HBV through vaccination, early detection, and effective treatment strategies.

Conflict of Interest

The authors declare that no conflict of interest.

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