

Research Article

Hepatitis B Virus_Surface Gene Mutations and their Clinical Implications

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Abstract

Hepatitis B infection is a major public health problem caused by hepatitis B virus (HBV). Factors associated with host immunity such as (HBV specific T- and/or B-cell) production and antigen presentation failure and viral determinants such as the HBV genotypes and their evolving variants, have largely contributed to and justified variations that occur in the HBV surface gene. Hepatitis B surface gene mutations may influence the accuracy of the results obtained with currently used serological diagnostic tests and may represent a great risk for the community, since neither hepatitis B vaccines nor hepatitis B immunoglobulin will prevent the infection by HBV. Out of 96 published papers from (1988 till 2016) downloaded from Google scholar and PubMed and evaluated according to the relevance of scientific data for the surface gene mutations of hepatitis B virus then 52 papers of them were selected and included in this study, then we reviewed and evaluated the current published papers about the surface gene mutations worldwide in which G145R represents the most common hepatitis B surface gene mutation reported in the literature. Furthermore, we reviewed their clinical implications and their impact on hepatitis B vaccination and treatment.

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الملخص

عدوى التهاب الكبد الفيروسي الوبائي هي مشكلة صحية عامة رئيسية ناجمة عن فيروس التهاب الكبد الوبائي. العوامل المرتبطة بمحصانة المضييف مثل انتاج الخلايا التائية والبائية المحددة لفيروس الكبد الوبائي وعدم تقديم المستضد ومحددات الفيروسية مثل المورثات لالتهاب الكبد الوبائي والمتغيرات المتطرفة له، ساهمت إلى حد كبير وبررت الاختلافات التي تحدث في الجين لسطح هذا الفيروس. الطفرات التي تحدث للمستضد السطحي لفيروس التهاب الكبد الوبائي قد تؤثر على دقة النتائج التي يتم الحصول عليها في اختبارات التشخيص المصلية المستخدمة حاليا وقد تمثل خطراً كبيراً على المجتمع، حيث لا لقاحات أو غلوبولينات مناعية لفيروس التهاب الكبد الوبائي تمنع العدوى عن طريق هذا الفيروس. من أصل ٩٦ من الأبحاث المنشورة في الفترة (١٩٨٨ حتى ٢٠١٦) تم تحميلها من باحث جوجل والبيب ميد تم تقييمها وفقاً لأهمية البيانات العلمية للطفرات الجينية لسطح فيروس التهاب الكبد الوبائي تم اختيار ٥٢ ورقة منها وادرجت في هذه الدراسة، ثم استعرضنا وقييمينا الأوراق المنشورة الحالية حول الطفرات الجينية لسطح فيروس التهاب الكبد الوبائي في جميع أنحاء العالم والتي مثل فيها الجين G145R الطفرة الأكثر شيوعاً من الطفرات الجينية لسطح فيروس التهاب الكبد الوبائي التي ذكرت في الأدب. وعلاوة على ذلك، استعرضنا آثارها السريرية وأثرها على العلاج والتطعيم ضد التهاب الكبد الوبائي.

1. Introduction

Hepatitis B is considered a life -threatening liver infection caused by hepatitis B virus. It affects about 350 million people around the world and it is associated with high risk of liver cirrhosis and hepatocellular carcinoma (HCC) [1]. Chronic hepatitis B infection is considered as an important healthcare problem worldwide due to the significant morbidity and mortality as well as being the cause of at least 50% of worldwide cases of hepatocellular carcinoma and approximately 30% of liver cirrhosis [2]. According to World Health Organization (WHO) more than 780,000 deaths occur worldwide annually due to chronic complications of HBV associated liver disease [3]. The prevalence of HBV carriers varies from 0.1% to 2% in low prevalence areas (United States and Canada, Western Europe, Australia and New Zealand followed by 3% to 5% in intermediate prevalence areas (Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America) and 10% to 20% in high prevalence areas (Southeast Asia, China, sub-Saharan Africa) [4]. The seroprevalence of HBsAg in central Sudan is 17.5% [5].

HBV genome is a circular double stranded DNA of full length negative strand (3020 – 3320) and incomplete positive strand (1600 – 2800). It is composed of four partially overlapping open reading frames (S, C, P and X) [6]. The S ORF encodes the surface envelope protein of the virus (HbsAg) and can be subdivided into pre S₁, preS₂ and S regions. As illustrated in Figure 1, the core gene (C gene) is divided into pre core

and core regions where P gene (polymerase protein) is about 800 amino acids that is divided into three domains. The X ORF protein domain (HbxAg) is involved in signal transduction, DNA repair and transcriptional activation. The mechanism of HbxAg is not completely understood but it may contribute to oncogenic activity of HBV [7]. Studies suggested that there are a lot of virus genotypic variants and associated spectrum of pathogenicity. Mutations in the S gene region occur under the selection of passive or active immunoprophylaxis and antiviral treatment or spontaneously [8].

The aim of this review was to highlight the most common mutations of HBV surface gene and their clinical impacts.

2. Material and Methods

PubMed (www.ncbi.nlm.nih.gov) and Google scholar was searched with keywords like "HBV", "Hepatitis B virus", "Hepatitis B virus surface gene mutations" and related words, to finalize this review article. Ninety-six research and review articles were selected. The selection criteria, of these research and review articles, were to include the data which represent the global burden of s gene mutations. The exclusion criteria were irrelevant scientific data. Finally, 52 papers selected and included in this study. This review done during the period of 4 months from Sep – Dec 2016.

2.1. Host Immune Response to HBV

Innate immunity eventually plays a role immediately after infection to limit the virus multiplication and initiates the development of an adaptive immune response. Innate host responses during the establishment of viral infections are mainly characterized by the production of cytokines such as type 1 interferon (IFN) α/b and the activation of natural killer (NK) cells. Production of type 1 IFNs can be induced directly by virus replication via cellular mechanisms that detect the presence of viral RNA or DNA. NK cells are activated by the recognition of stress-induced molecules and/or the alteration of the quantity of major histocompatibility complex (MHC) class I molecules on the surface of infected cells [9]. In adaptive immune response the antigen presenting cells kupffer cells and dendritic cells responsible for the activation of HBV specific T cells and production of interleukin 12(IL12) and TNF α , which induce IFN γ production and proliferation of CD8 $+$ T cells which is the main effector in HBV clearance. In contrast depleted CD8 $+$ T cells in acutely infected individuals associated with the persistence of HBV infection. The synergistic effect of cytokines and cytolytic activity of CD8 $+$ T cells allows for clearance of the virus without progressive liver damage and is consistent

with viral and lymphocyte kinetics noticed in chimpanzees following acute HBV infection. Activated T-helper cell type 2 (Th2) CD4+ T-cells shown to induce B-cell production of HBsAb, HBcAb and HBeAb in patients undergone HBV clearance [10, 11].

2.2. Virology and Genotypes

The HBV genome is a relaxed double-stranded circular DNA molecule of 3.2 kb in length [12]. It is classified into ten genotypes (A-J) that are scattered at different geographical areas and each has specific clinical outcome. Genotyping is usually done using different techniques such as line probe assay, genotype specific polymerase chain reaction and restriction fragment length polymorphism [13]. Acute infection with genotype D is considered as the main cause of acute liver failure than other genotypes [14] while genotype A appears to have more favorable outcome than genotype D [15]. Genotype C appears to have a significantly higher viral load than genotype B [16].

2.3. HBV Life Cycle

The replication process started with the entry of the HBV into the hepatocytes, then the virion undergoes uncoated process and transported to the nucleus in which RC DNA is converted into covalently closed circular DNA (cccDNA), cccDNA is transcribed into subgenomic RNA (sgRNA) and pregenomic RNA (pgRNA) [17, 18]. The subgenomic transcriptis used for the translation of envelope protein and X protein. The pgRNA is used as a template for reverse transcription, translation of HBcAg and HBV Pol [19]. In the cytoplasm the pgRNA is undergo reverse transcription to a nucleocapsid contain DNA which is enveloped to be secreted as progeny virion through the endoplasmic reticulum [20].

2.4. Epidemiology and Clinical Infection

The geographical distribution of HBV genotypes and HBsAg is as follows; Genotype A is found in North America and northwestern Europe, genotypes B and C are found in East Asia, genotype D is highly prevalent in the Mediterranean and the Middle East, genotype E identified in West Africa, genotypes F and H are most prevalent in Central and South America, and genotype G is found in the United States and Europe [21, 22]. In 2008 scientists isolated genotype I and the genotype J from Japanese patients [23, 24].

HBV is transmitted by exposure to infected blood or other body fluids. HBV transmission has been reported with several forms of human contact such as mother to child, household, sexual, needle stick and occupational/health care related. The highest concentrations of infectious HBV are found in blood and serum. However, other body

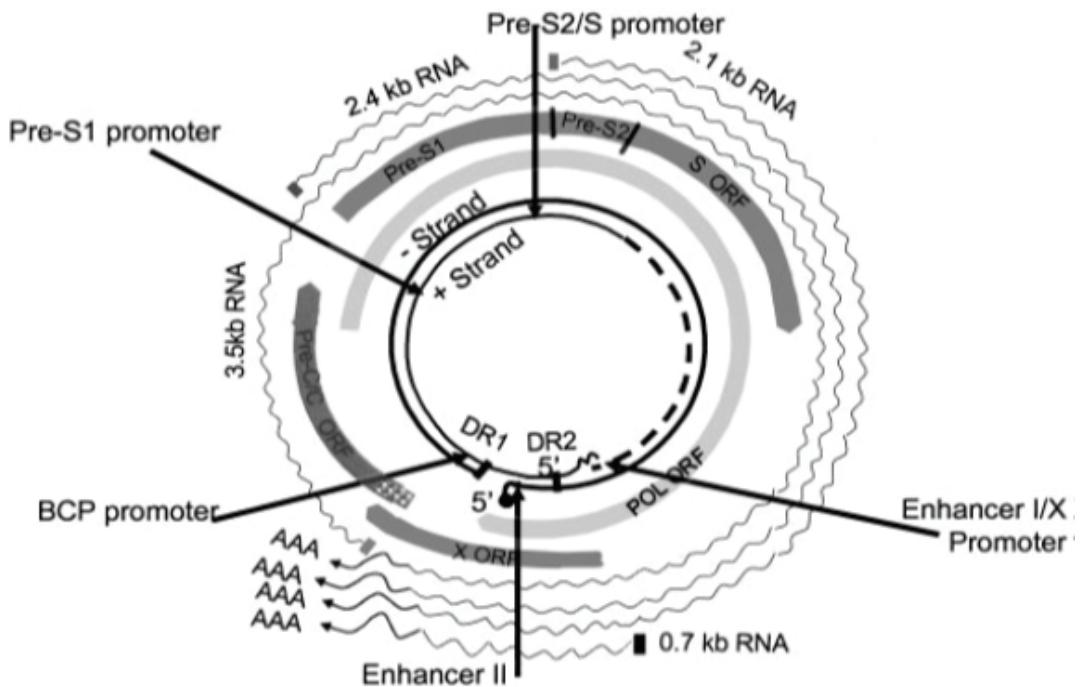


Figure 1: HBV genome showing open reading frames, major RNA transcripts and the regulatory elements of the virus [19]. With permission from Prof Stephen Locarnini.

fluids, such as semen and saliva, are also infectious [25]. HBV infection may result in subclinical or asymptomatic infection, acute self-limited infection, or fulminant hepatitis requiring liver transplantation. People infected with HBV can develop chronic HBV infection, which may lead to cirrhosis or hepatocellular carcinoma, the likelihood that newly infected individuals will develop chronic HBV infection is related to their age at the time of infection [26].

2.5. Hepatitis B Surface Antigen (HBsAg)

Hepatitis B surface antigen is a complex macromolecular structure that provides the envelope protein of HBV, it is composed of 75% proteins and 25% carbohydrates and host derived lipids. HBsAg produced during the infection of hepatocytes in the form of lipoprotein of 22nm diameter. The HBV envelop proteins composed of Pre-S1, Pre-S2 and S. The S protein, which codes for the S gene which is made up of 226 amino acids and is the main component of the viral envelop protein [27]. The three envelop proteins can be translated as L (large), M (middle) and S (small) or collectively HBsAg. Within the later the site between the aa 100-160 termed as major hydrophilic region (MHR). This region comprised of aa 99-160 refers to "a" determinant region. MHR proposed to have two major loops and one minor loop which defined by some disulphide bridge that join the epitope cluster for which majority of anti HBs directed are against the epitope between aa 107-137 or (138), 139-147 or(149) and 121-124 [28].

The "a" determinant is considered as the main neutralizing epitope and its common in all HBV genotypes, for this reason the anti HBs against the "a" determinant are widely protective against reinfection by HBV [29]. Changes in the conformation which can successively lead to failure of binding of neutralizing antibodies may develop due to amino acid substitutions within the "a" determinant [30]. Mutations in HBV occur due to low fidelity of polymerase, high replication rate and overlapping reading frames. The pre-S1/S2/S ORFs codes for three envelope proteins (large, middle and small) which are responsible for virus assembly and attachment to hepatocytes. L protein pre-S1 domain (amino acids 21-47) is the substrate for viral receptor attachment; M protein (pre-S2 domain) function is not well understood and, finally, S protein (S domain) is the HBsAg. Mutation in HBV is either vaccine induced or drug induced and these mutations can lead to occult hepatitis B infection, HBV reactivation and reinfection. Also it may lead to diagnostic assay failure [31]. HBsAg contains the epitope for "a" determinant region between the amino acid residue 99- 169 and amino acid changes in this site that lead to mutations that escape the immune response that provided by the vaccine [32].

2.6. Mutations in HBV Genes

Several published studies suggested that hepatitis B virus (HBV) mutants must be considered in occult HBV infection (OBI) which is characterized by the presence of HBV infection without detectable HbsAg. However, it is not known how widespread these mutants are and how they change the course of liver diseases. Jin lin hou et al. studied 2,565 individuals with chronic hepatitis B infection, hemodialysis patients, blood bank donors and cryptogenic liver cirrhosis. They found that 51 of them had occult hepatitis B infection and 43% of them had mutations in the MHR region includes the following Q101K, T115A, K122N, T123A, T126N, Q129N, G130R, T131I, M133T, F134L, C138Y, K141E, P142S, G145R, N146S, and C147F/R1 [33]. The existence of occult HBV patients caused by HBsAg mutants has implications for their possible transmission role through sexual contact and by blood transfusion. Naturally occurring mutations may envolve the polymerse gene as the tyrosine -methionine-aspartate-aspartate (YMDD-motif) mutations can occur spontaneously without using of antiviral therapy. Accumulation of base mismatch due to the natural features of viral polymerase might be the cause of this mutation [34].

2.7. Vaccine Escape Mutants

HBV vaccine is a recombinant DNA vaccine that contains HBsAg genetically engineered from the yeast *Sacchromyces Cerevisiae*. It provides seroprotection rate of 85 - 100 %

that seen one month after the last dose of vaccine and it confers immunity for at least 10 years [35]. Vaccine escape mutants at the "a" determinant region occurred under selection pressure of HBV vaccine administration or hepatitis B immunoglobulin (HBIG) or both [36]. The first HBsAg gene mutation was observed in vaccinated Italian child who was presented with both HBsAg and anti HBs. Gene sequencing showed substitution in which glycine replaced by arginine at site 145(G145R mutant) [37]. This G145R represents the most prevalent HBsAg mutation in the literature. It is addressed as a public health concern because of its capability of escaping the immune system and it found in immunocompromised patients and infant of HBeAg positive mother with prevalence of 3.1%. It may accompany other mutations such as T126I-T131A-C139Y-E/D144G, T126I-M133L, and P120Q-T126I in 37.5% [38]. Ngui et al. studied 17 HBV-infected mother and infant pairs as the infants became infected with HBV inspite of immunoprophylaxis administration. The S gene was sequenced for all patients and 15 mother/infant pairs showed complete concordance, while in the other two pairs showed the following: in one infant there were three nucleic acid changes (P120Q, F134Y and D144A) and the other harbored the I126N substitution, mutations that might interfere with HBsAg/anti-HBs binding [39]. Furthermore, multiple point mutations such as deletions and recombination were discovered in the preS region. Apart from the preS variants, a number of mutations had also been detected within the "a" determinant of the major hydrophilic region (MHR) of the surface antigen, against which natural or vaccine induced neutralizing antibodies are generated [40].

3. Abbreviations

V: vaccine, LMV: lamivudine, HBIG: hepatitis B immunoglobulin, A: alanine, R: arginine, D: aspartate, C: cystine, F: phenylalanine, G: glycine, H: histidine, I: isolucine, L: lucine, M: methionine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, W: tryptophane, Y: tyrosine.

HBV mutants remain stable over time and their transmission can occur horizontally or vertically [41]. Missense mutations within the "a" determinant were responsible 3.5% in 177 restaurant employee in China [42]. The high endimicity areas represent the most common regions in which vaccine escape mutants were abundant [43]. Monica et al. reported a case about a Caucasian man who develop acute hepatitis B infection inspite of having anti-HBs after vaccination, surprisingly this man acquired a mutant strain with diminished affinity for anti-HBs due to three amino acid substitutions discovered as follows (M125T, T127P and Q129H),these mutations were rarely reported together [44].

Amino Acid Position	Wild Type	Mutant	Cause
118	S	W	HBIG
120	S/P	Q/S	V-HBIG
125	M	T	HBIG
127	T	P	HBIG
128	A	V	LMV
129	Q	H	V-HBIG
133	M	L/I/T	V-HBIG
134	F/Y	N/R	HBIG
142	P	S	V
143	S	L	LMV
144	D	A/E	V-HBIG
145	G	A/R	V/LMV/HBIG
182	W	S	LMV
190	V	A	LMV
193	S	L	LMV
195	I	M	LMV
204	M	V/I	LMV

TABLE 1: The most studied HBV mutations: wild type, mutant type and the cause of mutations. Done by the authors [33, 38, 39, 44].

4. Drug Induced Mutations

The desired goal of treatment of CHB infection is to arrest the progression of liver injury and to improve the quality of life by preventing progression to cirrhosis, HCC and death. So far, eradication of the virus is impossible and current antiviral treatment aims to reduce liver failure and HCC and to increase survival, through the effective HBV DNA suppression [45]. United States Food and Drug Administration (FDA) has approved six drugs for the treatment of the CHB infection, of these immunomodulator (pegylated interferon), nucleos(t)ide analogues (lamivudine, entecavir, telbivudine, adifovir and tenofovir) [46]. Since the ORF encoding HBsAg overlaps with that of the polymerase, mutations within the former may affect the latter or vice versa, therapy with lamivudine results in several mutations in the polymerase gene, some of them are associated with alterations in the 'a' determinant of HBsAg [47]. Lamivudine is responsible for the highest rate of resistance reaching up to 70% by year 4 of continuous therapy [48]. Naturally occurring mutations are restricted to the "a" determinant region, whereas drug-associated mutations generally occur downstream to the MHR, Kazim et al. has studied 57 patients with histological proven CHB infection and were on lamivudine treatment of 100mg/day. Serum samples were taken at base line then after lamivudine therapy, DNA extracted and the region of MHR and flanking area were amplified and sequenced. The result showed that two patients (3.5%) have naturally occurring

mutations in the "a" determinant of the S gene and 24.5% mutations was lamivudine induced and mainly occurs downstream of the MHR and associated with corresponding mutations in the polymerase gene. LMV selected mutations downstream of "a" determinant considered as a cause of decrease in antigenicity of protein and binding to anti-HBs antibodies. In other recent studies, 8 out of 57 patients developed (rt204I/VDD) lamivudine resistant polymerase mutant that lead to surface gene mutations such as sW196Stop, sI195M and sW196L [49, 50].

Gloria Selabe et al. studied 17 patients with chronic hepatitis B infection who received 150mg/day lamivudine treatment at Johannesburg General Hospital between 1997 and 2004. Mutations in the YMDD region were determined in 13 patients, of those 7 carried rtM204I, 2 of them were hepatitis B e antigen(eAg) positive and 5 with eAg negative, and the remaining 6 showed rtM204V mutation, 4 eAg positive and 2 eAg negative. Additionally, the switching from rtM204I to rtM204V was reported in one patient after 24 months of therapy which indicates that Lamivudine resistance may develop at similar rates in HBeAg-positive and negative patients [51]. Recently it was concluded that there is no benefit in continuing lamivudine therapy after emergence of YMDD mutations [52].

5. Conclusion

The emergence of natural mutations should be expected due to the characteristics of the HBV genome such as proofreading capacity failure and viral factors such as the mechanisms of viral production and clearance. The mutations alter the binding of antibodies developed against wild-type S protein to virions and subviral particles and it can lead to diagnostic assay failure also. Most patients

with chronic hepatitis B had a good response to Lamivudine therapy but the long term therapy leads not only to emergence of lamivudine resistance or drug-resistant polymerase mutants, but also to the appearance of S gene mutants and YMDD mutants' attenuated efficacy of lamivudine treatment. We here propose to develop a vaccine which includes the most common mutations as well as wild type one and to build a strategy for the regime of chronic hepatitis B patients such as combination therapy.

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