REVIEW ARTICLE

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Hepatitis B e Antigen-Negative Chronic Hepatitis B

Maryam Vaez Jalali[1], Seyed-Moayed Alavian MD [2]

[1] PhD Student, Faculty of Public Health, Tehran University of Medical Sciences, Tehran Hepatitis Center, Tehran, Iran
[2] Associate Professor of Gastroenterology and Hepatology, Baqiyatallah University of Medical Sciences, Tehran Hepatitis Center, Tehran, Iran

Introduction

Hepatitis B virus (HBV) infection is a global health problem. Current estimates are that 2 billion people have been infected worldwide, of these, 360 million suffer from chronic HBV infection resulting in over 520 000 deaths from acute hepatitis B and 470 000 from cirrhosis or liver cancer[1]. The prevalence of hepatitis B carriers varies in different parts of the world, ranging from less than 1% to 15%. In the Middle East, the endemicity is intermittent, with a carrier rate of 2% to 7% [2].

It is estimated that over 35% of Iranians have been exposed to the HBV and about 3% are chronic carriers, ranging from 1.7% in Fars Province to over 5% in Sistan and Balouchestan[3].

To date, eight different genotypes of the HBV have been identified (A-H). The clinical spectrum of HBV infection ranges from subclinical to acute symptomatic hepatitis or, rarely, fulminant hepatitis during the acute phase and from the inactive HBV infection and chronic hepatitis of various degrees of histologic severity to cirrhosis and its complications during the chronic phase[4,5].

Thirty years ago, the diagnosis of chronic hepatitis B (CHB) was thought to require the presence of hepatitis B e antigen (HBeAg), as a reliable and sensitive marker of hepatitis B virus (HBV) replication. Individuals positive for hepatitis B surface antigen (HBsAg) but negative for HBeAg were considered to have non replicative HBV infection, and if their liver enzymes were normal or nearly normal they were referred to as asymptomatic or healthy HBsAg or HBV carriers. On the other hand, if they displayed elevated serum aminotransferases and liver histology indicative of chronic hepatitis, they were generally thought to be suffering from other superimposed or complicating conditions such as hepatitis D virus infection, alcohol-induced, metabolic, autoimmune, drug-induced, or other forms of chronic liver disease[6].

In the early 1980s it became apparent that HBV could replicate in the absence of HBeAg. Patients from the Mediterranean area, although negative for HBeAg and positive for antibodies to HBeAg (anti-HBe), were reported to have CHB with replicating HBV. The term anti-HBe-positive or HBe Ag negative CHB was then proposed and subsequently became widely accepted. In 1989 the molecular basis of this form of CHB was discovered with the identification of HBV mutations preventing HBeAg formation from an otherwise normally replicating HBV[7]. With time, it became apparent that HBeAg-negative CHB, initially viewed as an atypical and rare form of CHB mainly restricted in the Mediterranean area, had a much wider geographical distribution and that its frequency was increasing[6]. Its molecular virology and immunology were found to be more complex than initially thought[8], whereas the selection of precore HBV mutants was shown to be largely determined by the HBV genotype[9]. Mutations abolishing or diminishing HBeAg formation were identified along with changes in other parts of the HBV genome[9].

Overview of the hepatitis B genome and its mutational frequency

The hepatitis B virus is a small, DNA-containing

Correspondence:
Maryam Vaez Jalali, Department of Virology, Faculty of Public Health, Tehran University of Medical Sciences, Poorsina St., Enghelab Ave., Tehran, Iran

Tel: *+98 21 8896 2343*
Fax: *+98 21 8895 0595*

E-mail: *vaezjalali@razi.tums.ac.ir*

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virus with 4 overlapping open reading frames (i.e., several genes overlap and use the same DNA to encode viral proteins) (Fig. 1). The 4 genes are core, surface, X, and polymerase. The core gene encodes the core nucleocapsid protein (important in viral packaging) and hepatitis B e antigen. The surface gene encodes pre-S1, pre-S2, and S protein (yielding large, middle, and small surface proteins, respectively). The X gene encodes the X protein, which has transactivating properties and may be important in hepatic carcinogenesis. The polymerase gene encodes a large protein with functions critical for packaging and DNA replication (including priming, RNA and DNA dependent DNA polymerase, and RnaseH activities)[10]. Although HBV is a DNA virus, replication is through an RNA-replicative intermediate requiring an active viral reverse transcriptase/polymerase enzyme. The reverse

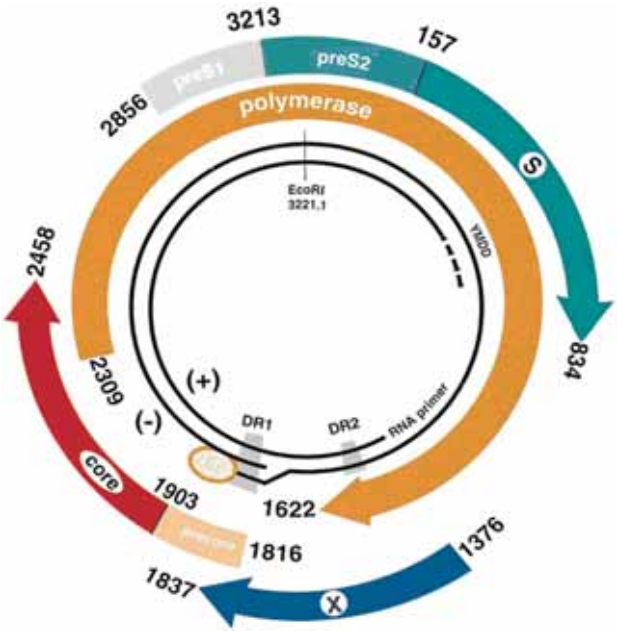

Figure 1. Organization of the HBV genome (genotype A). The inner circles represent the minus (2) and (1) DNA strands of the viral genome. The HBV polymerase is shown as an orange circle covalently bound to the 58-end of the (2) DNA strand. The nucleotide numbering of the genome is based on the unique EcoRI restriction enzyme site shown. The different open reading frames encoded by the genome, designated as S, core, polymerase, and X, are indicated by the arrows. Nucleotide numbers designate the boundaries of each ORF with position 1 mapped at the EcoRI site. Shown also are the map positions for the viral direct repeats (DR1 and DR2) and the approximate position of the YMDD locus in the HBV polymerase gene. Abbreviations: S, surface antigen; Y, tyrosine; M, methionine; D, aspartate; prec, precore; DR, direct repeat segment, used in viral replication.[9]

transcriptase (for both HBV and human immunodeficiency virus) is believed to lack a proofreading function that is common to other polymerases. Therefore, HBV exhibits a mutation rate more than 10-fold higher than other DNA viruses[11]; the estimated mutation rate is approximately one nucleotide/10,000bases/infection year. In addition, the accuracy of replication by the reverse transcriptase has been shown to vary with intracellular deoxynucleotide triphosphate concentrations[12].

Definition and nomenclature

e-CHB (or HBeAg-negative chronic hepatitis B): Patients with e-CHB are HBsAg-positive for at least 6 months, HBeAg-negative, anti-HBe-positive, with HBV DNA detectable in serum using unamplified assays, and active liver disease (elevated AST or ALT, liver histology showing chronic hepatitis with or without cirrhosis, or clinical evidence of cirrhosis)[13].

Prevalence

An estimated 350 million individuals in the world have chronic HBV infection[1;14]. Although positive for HBsAg, most of them are HBeAg negative. HBeAg positivity is highly prevalent only in younger age groups of HBsAg carriers[14]. The median prevalence of e-CHB among HBsAg-positive/HBeAg-negative patients was 32%, with the highest median prevalence in Asia Pacific (36%) and lower prevalences in the Mediterranean (24%), the United States, and Northern Europe (22%). Differences in prevalence of e-CHB in different regions of the world are in part related to the geographical variation in HBV genotypes[13].

In HBV genotype A, cytosine is present at position 1858 (C-1858) precluding the selection of the G1896A mutation (Figure 2) [15]. This explains the low frequency of precore mutants in Northern

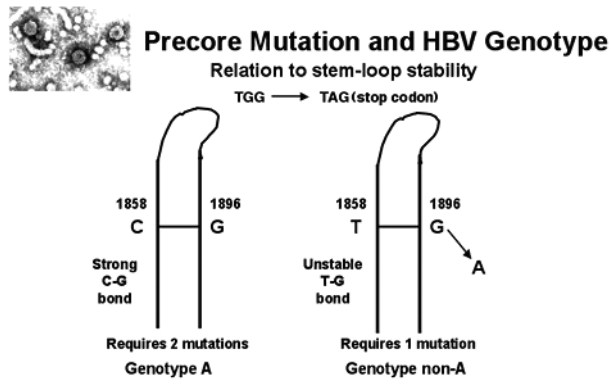

Figure 2. Precore mutation and HBV genotype

Europe, North America, and parts of Africa where genotype A predominates [16]. In contrast, the non-A HBV genotypes (B, C, D, and E) harbor thymidine at the same position (T-1858), which pairs with A at 1896 [16]. Thus, precore mutants prevail in the Mediterranean where non-A genotypes, particularly D, are predominant[17;18].

In Iran, there is a new report about HBV genotypes in 26 patients from a referral hospital. S and C regions sequencing showed that all these patients had HBV D genotype [19]. It is estimated that almost 58% of HBV infections in Iran are precore mutants [20]. The great majority of HBsAg positive/HBeAg negative individuals have normal ALT values. However, a number suffer from CHB (15%-30%) [6;21]. The prevalence of e-CHB seems to vary geographically. Possible contributing factors for its development include vertical transmission of HBV, long duration of infection and male sex [6;14]. In previous studies, only a few countries were found to have more HBeAg-negative than HBeAg-positive chronic hepatitis B [22] but now it is apparent that there is a worldwide increase in the prevalence of e-CHB [14]. In Italy, 41% of patients with CHB during the period between 1975 and 1985 were HBeAg negative but in the last decade this has increased to 90% [21].

Emergence and selection of HBeAg-negative

HBV mutants

Once immune pressure to the wild-type virus starts to mount, selection of HBeAg-negative mutants and their predominance over the wild-type HBV is hastened [14;23]. But it remains to be determined why HBV mutants that are not producing HBeAg would be privileged to become selected over the wild-type virus during or after HBeAg loss and seroconversion. It is becoming clear that in the absence of immune-mediated hepatocyte damage, HBeAg-negative mutants are not selected[14;23;24]. This is best illustrated by HBV genome analyses in patients with persistently high viral replication and normal aminotransferases (indicating absence of liver injury) during the HBeAg positive phase, where only few, if any, mutations are detectable[25]. Therefore, their selection is not a primary event implicated in the loss of tolerance to HBV but most likely secondary to the already-mounted immune response against HBV. HBeAg-negative mutants most likely exhibit certain biological properties that render them less vulnerable to host immune reactions compared with wild-type HBV [6].

Mutations in e-CHB (HBeAg-negative chronic hepatitis B)

The most commonly studied mutation associated with e-CHB is in the pre-core region at nucleotide (nt) 1896 where adenine (A) is substituted for guanine (G), producing a stop codon that prematurely terminates synthesis of the HBeAg [7]. The core promoter region regulates transcription of the pre-core region. Therefore certain mutations in this region can affect HBeAg synthesis. Specifically, a double mutation involving substitution of T for A at nt 1762 and A for G at nt 1764 can reduce pre-core mRNA and HBeAg production [26-28].

e-CHB and disease severity

The relationship between precore/core promoter variants, serum HBV DNA levels, and severity of liver disease is unclear. Some [26;28;29] but not all [30] in vitro studies suggest that core promoter mutations increase HBV replication.

The significant nucleotide and amino acid divergence in the core promoter and precore region and its link to hepatitis B disease activity is well documented in some studies for example Bozdaie et al31 suggested that an active immune response of the host to viral epitopes localized in core promoter region may play an essential role and may thus have clinical significant[32-35]. But some studies [36] found that no significant correlation exists between core promoter and precore mutations, viral replication and liver damage in chronic hepatitis B infection.

Briefly different studies found different results. These findings suggest that geographical differences represented possibly by both viral (e.g. the predominant HBV genotype) and host factors (e.g. HLA type?) may influence the occurrence of these mutations [31].

In 2002, Funk et al reviewed 50 of 281 research articles potentially related to e-CHB by a literature search; and suggested some major sources of variability in these articles. Apart from the geographical location and predominant HBV genotype, various factors may affect the prevalence of e-CHB including gender, age, treatment history, and duration of infection. Because all of these factors vary between studies, comparing the results of one study with another or combining the results of several studies from the same region may not be appropriate. In order to better understand the complex interplay between these factors and the development of e-CHB, sufficiently large studies

using multivariate techniques to adjust for these potential confounders are needed [13].

It is important to know whether determination of precore and core promoter mutations may help in predicting the different outcomes along the course of HBeAg to anti HBe seroconversion [37]. But to date no clinical or virological factor has been identified which accurately predicts the risk of disease in patients with e-CHB.

Management

Some studies suggest that the HBeAg negative variants may be more resistant to the immune clearance actions of interferon and is consistent with the hypothesis that the HBeAg negative strains emerge because of immune selection [38].

Basal core promoter and pre-core mutations in the HBV genome enhance replication efficacy of lamivudin resistant mutants [39;40]. Also one study showed that the precore stop codon mutation appears to compensate for the decreased replication phenotype of the lamivudin mutants In Vitro[41]. The newly approved adefovir has been tested in e-CHB and it appears to have a similar efficacy as in HBeAg positive infection [42].

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