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World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants

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SUMMARY. Hepatitis B is a serious disease that is endemic in many parts of the world. A significant proportion of patients with chronic hepatitis B (CHB) are infected with a variant form of the hepatitis B virus (HBV) which decreases or abolishes the production of hepatitis B e-antigen (HBeAg). The purpose of this literature review is to describe the epidemiology of HBeAg-negative CHB (e-CHB) worldwide. A literature search was conducted to identify studies pertaining to e-CHB and underlying variants (precore and core promoter). Fifty studies were included in our analysis. The median prevalence of e-CHB among patients with chronic HBV infection was 33% in the Mediterranean, 15% in Asia Pacific, and 14% in the USA and Northern Europe. The pre core stop codon variant was detected in a median of 60% (range 0–100%) of HBeAg-negative patients overall, 92% in the Mediterranean, 50% in Asia Pacific and 24% in the USA and Northern Europe. There were very few data on the prevalence of core promoter variants outside Asia where the median prevalence among HBeAg-negative patients was 77%. This literature review revealed that e-CHB is more common than previously suspected and that it is present worldwide with marked variations in the prevalence of associated HBV variants across different geographical regions. Additional research using population based samples of adequate size based on a consensus definition of e-CHB and using standardized HBV DNA assays is needed to better estimate the true prevalence of e-CHB and its associated HBV variants.

Keywords: chronic hepatitis B, core promoter, epidemiology, precore.

INTRODUCTION

Approximately 350 million individuals world-wide are carriers of hepatitis B virus (HBV) [1]. Chronic HBV infection is the cause of up to 50% of cirrhosis and 70–90% of cases of hepatocellular carcinoma (HCC) in China, South-east Asia and Africa [2,3]. It is estimated that during 1990, 229 000 HCC cases world-wide were attributable to HBV infection [4].

Among the 350 million HBV carriers, 7–30% are believed to be infected with HBV variants that express little or no hepatitis B e antigen (HBeAg) [5]. The typical course of hepatitis B infection involves an HBeAg-positive phase with high serum HBV DNA levels. Subsequently, patients undergo a process of seroconversion in which HBeAg is lost and antibodies to HBeAg (anti-HBe) appear. Generally this signals the decline of HBV DNA to levels that are not detectable by unamplified assays and a return of aminotransferase to normal values. Among some patients, for reasons that are not yet clear, the immune pressure associated with seroconversion selects for HBV variants that express little or no HBeAg. Although the patient may develop anti-HBe, active HBV DNA replication continues with associated liver damage [5].

There are a variety of mutations in the core promoter and pre-core region that can decrease or prevent the synthesis of HBeAg without adversely affecting the ability of the HBV to replicate. The clinical syndrome in which patients are hepatitis B surface antigen (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 manifested by elevated aminotransferase (AST or ALT), liver histology showing chronic hepatitis with or without cirrhosis or clinical evidence of cirrhosis; will be referred to as

Abbreviations: CHB, chronic hepatitis B; e-CHB, HBeAg-negative chronic hepatitis B; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

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HBeAg-negative chronic hepatitis B (e-CHB) [6]. 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 [7,8]. 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 [9–11].

The primary aim of this literature review is to describe the epidemiology of e-CHB and the relative prevalence of the associated precore and core promoter variants.

METHODS

Data sources

The following terms were used to search Medline (1966 – April 2000) and PubMed (1966 – April 2000): 'precore' OR 'pre-core' OR 'pre-C' OR 'core promoter' AND 'hepatitis B'.

Articles identified by searches of Medline and PubMed were evaluated according to the following inclusion criteria:

Sample size: N > 40, except in instances where the only data available for a given country is from studies of fewer than 40 subjects.

Evidence of chronic infection: Subjects were HBsAg-positive for at least 6 months.

Methodology:

- Adequate detail in the description of the study population to determine the country from which the sample was drawn.
- For clinical trials, pretreatment data on the presence of e-CHB and HBV variants were reported.
- Determinations of HBV serology, liver disease (ALT, liver histology or clinical presentations) and HBV DNA were required to assess the prevalence of e-CHB.

The reference list for each article was reviewed to identify additional publications that were not identified via Medline or PubMed.

Definitions

HBsAg-positive (or chronic HBV infection)
Patients who were HBsAg-positive for at least six months fall into this category, no determination was made on clinical disease process. This term was used as a denominator to reflect the proportion with e-CHB among patients with chronic HBV infection (or HBsAg-positive).

HBeAg-negative
All patients who were HBsAg-positive but HBeAg-negative fall into this category, without determining clinical disease process. This term was used as a denominator to reflect the proportion with e-CHB among all the patients with chronic HBV infection (HBsAg-positive), who were HBeAg-negative. Some studies reported only the anti-HBe status of patients. Anti-HBe-positive patients were assumed to be HBeAg-negative for the purposes of our evaluation.

e-CHB (or HBeAg-negative chronic hepatitis B)
Patients with e-CHB were identified based on the serological and clinical information presented in each study reviewed. Patients with e-CHB were 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).

Prevalence estimates

The methods used to assess prevalence of e-CHB in each of the studies reviewed are described in the Results section. Because the sensitivity of assays used to detect HBV DNA will affect prevalence estimates, the assay used in each study is described in the table footnotes. Prevalence estimates were not adjusted for differences in age or gender distribution, proportion of patients who were HBeAg-positive, or proportion with active or advanced liver disease. The estimates of pre-core and core promoter variant prevalence include all HBeAg-negative patients with or without active liver disease.

The median prevalence of e-CHB among HBeAg-negative patients may be lower than the median prevalence of e-CHB among HBsAg-positive patients due to the fact that different studies contribute to the calculation of each median.

RESULTS

Two hundred and eighty-one research articles potentially related to e-CHB were identified by the literature search; 50 of these articles were included in our analysis. Two hundred and thirty-one were rejected primarily due to inadequate sample size, the use of a study population that was not generalizable (such as patients with liver transplants or hepatocellular carcinoma), or a patient selection process that focused on acute or fulminant hepatitis rather than chronic hepatitis.

Asia Pacific

The results from 26 studies conducted in the Asia Pacific region which include 6161 patients are summarized in Table 1. The data from two studies (one from Taiwan, another from Korea) included only hospitalized patients. The remainder of the data came from clinic populations (15 studies) or from an unknown but presumably clinic setting (8 studies). The mean age of the study populations

Table 1 Prevalence data from Asia Pacific

Country	*N*	Active disease (%)	HBeAg−ve (%)	HBV DNA+ (%)	% e-CHB of HBsAg+	% e-CHB of HBeAg−	% PreC+ of HBeAg−	% CP+ of HBeAg−
Japan								
[19]	227	nr	37(e−)	55[k]	8	18	nr	nr
[42]	61	84[c]	25(a+)	nr	nr	nr	100	nr
[43]	273	62[d]	58(e−) 47(a+)	77[j]	31	54	42	nr
[44]	55	56[d]	45(e−)	nr	24[f]	52	52	nr
[20]	93	51[c]	49(e−)	nr	17	35	100	98
[21]	42		74			40[g]	90	71
[22]	169	100	49(e−)	nr	nr	nr	nr	87
[45]	103	76[c]	55(a+)	nr	nr	nr	23	79
[10]	113	nr	53(e−)	nr	nr	nr	43	77
Mainland China								
[46]	54	100	nr	nr	nr	nr	63	nr
[47]	77	nr	100(e−, a+)	66[j]	nr	66[a]	48	nr
[48]	446	nr	45(a+)	53[j]	16[a]	35[a]	19	nr
[49]	177	50[c]	53(e−)	84[j]	26[b]	49[b]	57	59
Hong Kong								
[23]	308	49	35(e−)	85[j]	10[c]	29[c]	22	nr
[6]	350	45	69(e−)	39[m]	12[d]	17[d]	37	65
Taiwan								
[50]	79	42[d]	32(e−)	86[k]	15[d/k]	48[d/k]	nr	nr
[51]	94	100[c]	100(a+)	36[k]	nr	36[g]	nr	nr
[52]	2582	38	56(e−)	nr	12[e]	21[e]	nr	nr
[53]	62	53[d]	44(e−)	nr	21[h]	48[h]	93	nr
Korea								
[54]	66	nr	48(e−)	nr	nr	nr	80	nr
[55]	57	91[d]	56(e−)	96[j]	nr	nr	50	nr
India								
[56]	72	100	54(e−)	61[k]	15	28	nr	nr
[57]	120	nr	nr	nr	15	nr	nr	nr
Singapore								
[58]	404	nr	56(e−)	42[k]	5[f]	9[f]	nr	nr
Thailand								
[59]	34	74[d]	68(e−)	65[j]	47[i]	69[i]	nr	nr
Malaysia								
[60]	43	28[c]	33(e−)	100[j]	nr	nr	21	nr

nr, not reported; e−, % HBeAg-negative; a+, % anti-HBe-positive; PreC, precore variant; CP, core promoter variant; u, unknown. [a]HBV DNA+ and HBsAg+ > 6 month. [b]Elevated ALT and liver histology showing hepatitis with or without cirrhosis. [c]Elevated ALT. [d]Chronic active hepatitis with or without cirrhosis. [e]HBeAg−, ALT > 1.5 times ULN, DNA+. [f]ALT > 1.5x ULN, HBV DNA+. [g]Anti-HBe+, ALT > 2 times ULN (both inclusion criteria), HBV DNA+ by spot hybridization. [h]HBeAg− and diagnosis of chronic active hepatitis. [i]HBeAg−and ALT > ULN. [j]PCR amplified DNA; [k]Hybridization assay; [l]> 10^5 copies/mL; [m]Branched DNA assay.

ranged from 28 to 43 years with a mean of 35.5 years. Of the 18 studies that reported on the gender composition of the patient population, the percentage of females ranged from 3 to 46 with a mean of 27%.

The median prevalence of e-CHB among HBsAg-positive patients was 15% (range: 5–47) (Table 1). The median prevalence of e-CHB among HBeAg-negative patients was 36% (range: 9–69). The median prevalence of pre-core

variants among HBeAg-negative patients was 50% (range: 19–100) and the median prevalence of core promoter variants among HBeAg-negative patients was 77% (range: 59–98).

Mediterranean

The results from 17 studies conducted in the Mediterranean which include 1374 patients are summarized in Table 2. The data from two studies (both from Israel) were derived from samples analysed by diagnostic laboratories. The remainder of the data came from clinic populations (10 studies) or from an unknown but presumably clinic setting (5 studies). The mean age of the study populations ranged from 32 to 54 years with a mean of 40.8 years. Of the 12 studies that reported on the gender composition of the patient population, the percentage of females ranged from 13 to 33 with a mean of 25%.

The median prevalence of e-CHB among HBsAg-positive patients was 33% (range: 10–72) (Table 2). The median prevalence of e-CHB among HBeAg-negative patients was 24% (range: 0–84). The median prevalence of precore variants among HBeAg-negative patients was 92% (range: 67–100) and the single study from Turkey of core promoter variants reported a prevalence of 0%.

USA/Northern Europe

The results from seven studies conducted in the United States and Northern Europe that include 712 patients are summarized in Table 3. The data from two studies (both German) were derived from samples analysed by diagnostic laboratories and one French study reported on hospitalized patients. The rest of these studies evaluated patients from clinic settings. The mean age of the study populations ranged from 38 to 51 years with a mean of 41.5 years. Of the

Table 2 Prevalence data from the Mediterranean

Country	*N*	Active disease (%)	HBeAg− (%)	HBV DNA+ (%)	% e-CHB of HBsAg+	% e-CHB of HBeAg−	% PreC+ of HBeAg−	% CP+ of HBeAg−
Italy								
[61]	116	71[d]	54(e−)	nr	38[f]	70[f]	nr	nr
[62]	106	28[d]	100(a+)	26[b]	nr	17	nr	nr
[63]	92	64[d]	30(e−, a+)	80[b]	10	32	nr	nr
[64]	118	22[d]	100(a+)	24[b]	nr	19	nr	nr
[65]	42	60[d]	100(a+)	100[a]	nr	nr	88	nr
[66]	106	100[c]/71[d]	60(a+)	100[a]	nr	nr	95	nr
[27]	115	100[c]/71[d]	50(a+)	100[a]	nr	nr	97	nr
[67]	41	85[c]	71(a +)	100[a]	nr	nr	86	nr
Greece								
[68]	33	68[d]	76(e−)	64[b]	33	44	nr	nr
[69]	36	86[f]	86(e−)	86[b]	72[e]	84[e]	100	nr
Spain								
[70]	99	80[c]/81[d]	63(a+)	47[b]	15[b/c]	24[b/c]	nr	nr
[71]	45	100[c]	100(a+)	0[b]	nr	0	67	nr
[25]	42	88[c]/71[d]	55(a+)	69[b]	nr	nr	96	nr
Israel								
[72]	153	nr	100(e−)	7[b]	nr	7	nr	nr
[73]	120	nr	100(e−)	8[b]	nr	8	100	nr
Bulgaria								
[74]	29	69[d]	100(a+)	38[g]	nr	34[d/g]	89	nr
Turkey								
[75]	81	nr	42(e−)	100[a]	nr	nr	85	0

nr, not reported; e−, % HBeAg-negative; a+, % anti-HBe positive; PreC, precore variant; CP, core promoter variant.
[a]PCR amplified DNA. [b]Hybridization assay. [c]Elevated ALT. [d]Chronic active hepatitis with or without cirrhosis. [e]HBeAg−, ALT > 1.5 times ULN, HBV DNA+. [f]ALT > 1.5 ULN, HBV DNA+. [g] $\geq$ 50 pg/200 mL.

Table 3 Prevalence data from the USA and Northern Europe

Country	*N*	Active disease (%)	HBeAg− (%)	HBV DNA+ (%)	% e-CHB of HBsAg+	% e-CHB of HBeAg−	% PreC+ of HBeAg−	% CP+ of HBeAg−
USA								
[76]	33	58[c]	100(e−, a+)	30[i]	nr	30	24	nr
[77]*	45	64[d]	9(e−)	89[g]	nr	nr	0	nr
Germany								
[33]	96	100[a]	33(e−)	100[g]	nr	nr	53	59
[78]	127	nr	100(a+)	54[f]	nr	nr	16	nr
[79]	93	nr	84(e−, a+)	27[h]	13e	15e	40	nr
France								
[80]	276	100[b]	22(e−, a+)	100[g]	22	nr	nr	nr
Sweden								
[81]	42	40[c]	67(e−)	65[f]	14	22	nr	nr

nr, not reported; e−, % HBeAg-negative; a+, % anti-HBe positive; PreC, precore variant; CP, core promoter variant; u, unknown. *Included patients from the USA and UK. [a]DNA+ and HBsAg+ > 6 month. [b]Elevated ALT and liver histology showing hepatitis with or without cirrhosis. [c]Elevated ALT. [d]Chronic active hepatitis with or without cirrhosis. [e]HBV DNA+ (> 10^5 copies/mL). [f]PCR amplified DNA. [g]Hybridization assay. [h]> 10^5 copies/mL. [i]Branched DNA assay.

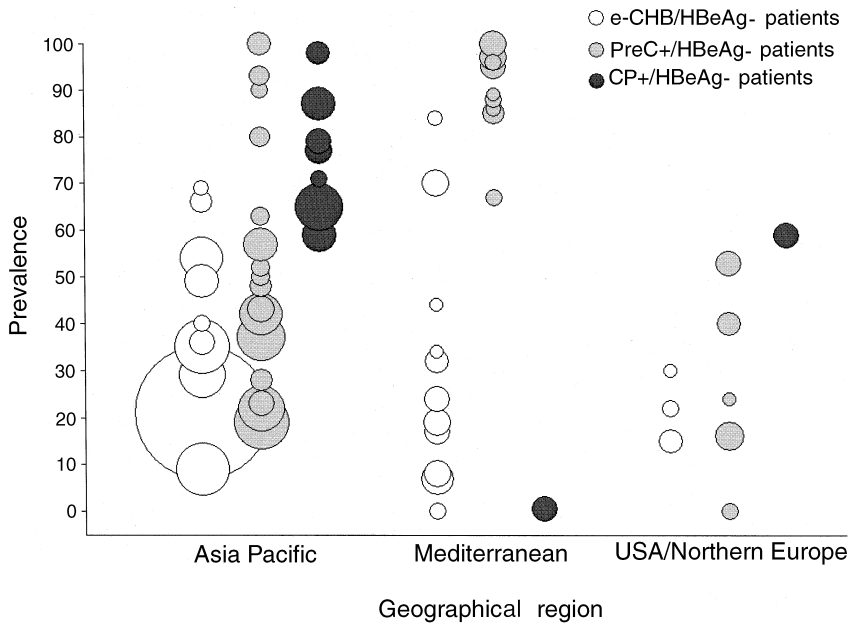

Fig. 1 Estimates of the prevalence of e-CHB, precore and core promoter variants among HBsAg-positive/HBeAg-negative subjects grouped by region. †Bubble size is proportionate to the sample size of the study population.

five studies that reported on the gender composition of the patient population, the percentage female ranged from seven to 26 with a mean of 18.2%.

The median prevalence of e-CHB among HBsAg-positive patients was 14% (range: 13–22) (Table 3). The median prevalence of e-CHB among HBeAg-negative patients was 22% (range: 15–30). The median prevalence of precore variants among HBeAg-negative patients was 24% (range: 0–53) and the single study from Germany of core promoter variants reported a prevalence of 59%.

Global prevalence of e-CHB, precore and core promoter variants

The bubble graph shown in Fig. 1 provides a synthesis of the prevalence data for e-CHB, precore and core promoter

variants among HBsAg-positive, HBeAg-negative subjects grouped by region. The size of each bubble is proportionate to the size of the study population.

DISCUSSION

Noncomparability due to differences in definitions and measurement

In addition to demographic differences in study populations described above that would be expected to influence the prevalence of e-CHB, there are two major sources of variability in the data collected that may contribute to noncomparability between estimates even if the underlying prevalence was the same. The lack of a standardized definition of e-CHB, or chronic hepatitis for that matter, makes it very difficult to compare the results across studies. Some studies defined e-CHB based on elevated ALT alone, others based their findings on DNA tests, and yet others utilize a combination of biochemical, histological and/or clinical assessment. This highlights the need for a consensus definition of e-CHB [12].

The second major source of variability is the lack of standardized HBV DNA assays and increased sensitivity of HBV DNA assays over time. As a result, a patient previously considered to have undetectable serum HBV DNA using hybridization assays with sensitivity limits of approximately 1 million copies/mL would now be considered to have ongoing HBV replication based on the results of polymerase chain reaction (PCR) assays which can detect a few hundred copies/mL of HBV DNA. In order that results from different studies can be compared, the type of assays used to detect serum HBV DNA, and their sensitivity and range of linearity must be reported. In addition, all HBV DNA assays should be standardized against a panel of international standards.

Another hurdle in the understanding of the literature on e-CHB is that some researchers equate the clinical syndrome of e-CHB with the presence of the precore stop codon mutation ($G_{1896}A$) despite the fact that the predominant HBV strain in these patients may be: (i) wild type in the pre-core and core promoter regions; (ii) precore stop codon variant; (iii) dual core promoter variant; (iv) combination of precore stop codon and core promoter variant; or (v) variants with other nonsense mutations in the precore region. Future research needs to report phenotypic as well as genotypic data to determine if the clinical outcome and response to treatment of patients with e-CHB correlate with the predominant sequence in the precore and core promoter regions.

e-CHB prevalence

The results from this literature review suggest that e-CHB is more common than previously suspected, that it can be found world-wide, and that it is not invariably associated with precore mutation (Fig. 1). The median prevalence of e-CHB among HBsAg-positive patients was 33% in the Mediterranean, 15% in Asia Pacific, and 14% in the United States and Northern Europe. Although e-CHB is more common in the Mediterranean, the impact is greatest in China where a prevalence of 15% among HBsAg carriers translates into approximately 15 million cases of e-CHB.

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%) and the United States and Northern Europe (22%). The differences in prevalence of e-CHB in different regions of the world are in part related to the variability in study design and sample selection, but an important explanation is the geographical variation in HBV genotypes [13–16].

Experience of clinical investigators in many parts of the world suggests that the prevalence of e-CHB is increasing over time [17,18]. This clinical impression is supported by data from a few studies. A study conducted in Japan between 1981 and 1984 found that 8% of HBsAg-positive patients met the definition of e-CHB [19]. A decade later, three studies show that the percentage has at least doubled [20–22]. Preliminary data from Italy also indicate that e-CHB is on the rise. Nevertheless, definitive evidence in support of an increase in prevalence of e-CHB in the last decade is lacking because of the scarcity of population-based studies.

Our findings contradict the conventional wisdom that e-CHB is not common outside of the Mediterranean. The belief that e-CHB is a Mediterranean problem may stem from the fact that research on this phenomenon originated in that part of the world. At the same time, these data indicate that e-CHB is not as common in the Mediterranean as recent anecdotal reports of e-CHB rates of 80% among HBsAg carriers in Italy would suggest. The lower rates reported here may reflect differences in the sensitivity of the assays used to detect HBV DNA or in the proportion of the study populations with active disease. It is also possible that there is a genuine increase in prevalence of e-CHB during the last 15 years that is not reflected in the published literature.

HBeAg-negative variants

The pre-core mutation ($G_{1896}A$) can be detected in 20–95% of HBeAg-negative patients in most areas, but the prevalence is highest in the Mediterranean countries (> 85%). Geographic variation in the prevalence of the precore variant is related to the fact that the occurrence of the $G_{1896}A$ mutation is restricted to HBV genotypes with T at nucleotide position 1858. Thus, the $G_{1896}A$ precore variant is most prevalent in HBV genotype D, the predominant genotype in the Mediterranean countries, intermediate in genotypes B and C, the most frequent genotypes in Japan and Southeast Asia, and rare in genotype A, the most

common genotype in North America and Northern Europe [14,23–25].

Unlike the precore mutation, the core promoter mutations $A_{1762}T$ and $G_{1764}A$ can be found in HBV genotypes with C at nucleotide 1858. In fact, there is some evidence that the core promoter mutations are more likely to be selected in genotypes with a C rather than T at 1858 [26]. These findings suggest that the geographical distribution of core promoter and pre-core variants may be different, and e-CHB may not be limited to the Mediterranean and Asia (Fig. 1). Our study showed that e-CHB has been reported worldwide and core promoter variants are common in Asia Pacific but very little data about the prevalence of core promoter variants in other regions of the world are available.

Population-based evidence

Nearly all of the studies reviewed were conducted in hospital-based clinic populations which we would not expect to be representative of all HBsAg carriers. In addition, disease severity varied across the study populations. Moreover, very few were cross-sectional studies that enrolled consecutive patients. Rather, most studies were retrospective and likely to be biased towards patients with more severe liver disease. Thus, our prevalence estimates based on review of existing literature have major limitations.

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.

Clinical implications

Several studies found that treatment of e-CHB with interferon is more difficult because of a high rate of relapse after treatment is stopped [27–30]. The exact reason for the low rate of sustained response is not clear. Studies that examined the correlation between precore and core promoter variants and response to interferon treatment have yielded conflicting results [27,29,31]. One study found that e-CHB patients with precore stop codon variant comprising > 20% of the viral population were less likely to have a sustained response to interferon treatment [27]. However, another study of HBeAg-positive Chinese patients treated with interferon found that those with the precore stop codon mutation were more likely to clear HBeAg and to do so earlier [32]. A recent study by Erhardt *et al.* [33] reported that among HBeAg-negative patients, core promoter mutations were associated with higher HBV DNA levels and poorer sustained response to interferon but the converse was true for HBeAg-positive patients. In that study, precore mutation had no correlation with response to treatment.

Clinical trials showed that lamivudine induces effective suppression of HBV replication leading to normalization of aminotransferases and significant improvements in histology, indicating that the responses among e-CHB patients are similar to those seen in studies of HBeAg-positive chronic hepatitis B patients [34–40]. However, most patients relapse after treatment is stopped [37]. Research continues to determine response rates to longer courses of lamivudine treatment and the durability of response post treatment. One study suggested that patients with core promoter variants are more likely to develop breakthrough infection [41], but additional studies are needed to determine if there is a correlation between response to lamivudine and core promoter or pre-core mutations.

CONCLUSIONS

Our literature review showed that e-CHB is present in Asia, the United States, Northern Europe and the Mediterranean and may be increasing. There are variations in the prevalence of e-CHB and the prevalence of precore and core promoter variants among HBeAg-negative patients in different parts of the world. However, absence of population-based studies, lack of standardized definition of e-CHB and variations in HBV DNA assays preclude definitive conclusion on the true prevalence of e-CHB and the contribution of precore and core promoter variants to e-CHB in each geographical region.

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REFERENCES

1 World Health Organization. Hepatitis B. Fact sheet. http://www.who.int/inf-fs/en/fact204.html, Accessed: 12 December 2000.
2 Lok ASF. Natural history and control of perinatally acquired hepatitis B virus infection. *Dig Dis* 1992; 10: 46–52.
3 Fattovich G. Progression of hepatitis B and C to hepatocellular carcinoma in Western countries. *Hepatogastroenterology* 1998; 45: 1206–1213.
4 Parkin DM, Pisani P, Muñoz N, Ferlay J. The global health burden of infection associated cancers. In: Newton R, Beral V, Weiss RA, eds. *Infections and Human Cancer.* Plainview, New York: Cold Spring Harbor Laboratory Press, 1999: 5–33.
5 Hadziyannis SJ. Hepatitis B e antigen negative chronic hepatitis B. From clinical recognition to pathogenesis and treatment. *Viral Hep Rev* 1995; 1: 7–36.
6 Chan HLY, Leung NWY, Hussain M, Wong ML, Lok ASF. Hepatitis B e antigen-negative chronic hepatitis B in Hong Kong. *Hepatology* 2000; 31: 763–768.

7 Carman WF, Jacyna MR, Hadziyannis S *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; 2: 588–591.

8 Brunetto MR, Stemler M, Bonino F *et al.* A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. *J Hepatol* 1990; 10: 258–261.

9 Moriyama K, Okamoto H, Tsuda F, Mayumi M. Reduced precore transcription and enhanced core-pregenome transcription of hepatitis B virus DNA after replacement of the precore-core promoter with sequences associated with e antigen-seronegative persistent infections. *Virology* 1996; 226: 269–280.

10 Takahashi K, Aoyama K, Ohno N *et al.* The precore/core promoter mutant ($T^{1762}A^{1764}$) of hepatitis B virus: Clinical significance and an easy method for detection. *J Gen Virol* 1995; 76: 3159–3164.

11 Buckwold VE, Xu Z, Chen M, Yen TSB, Ou J-H. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J Virol* 1996; 70: 5845–5851.

12 Hoofnagle JH, Lok A, Heathcote J. Standardization of nomenclature for hepatitis B. In: *Workshop on Management of Hepatitis B 2000.* NIH, Bethesda, MD: National Institutes of Health, 2000.

13 Li J-S, Tong S-P, Wen Y-M, Vitvitski L, Zhang Q, Trépo C. Hepatitis B virus genotype A rarely circulates as an HBe-minus mutant: Possible contribution of a single nucleotide in the precore region. *J Virol* 1993; 67: 5402–5410.

14 Lindh M, Andersson A-S, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus – Large-scale analysis using a new genotyping method. *J Infect Dis* 1997; 175: 1285–1293.

15 Norder H, Hammans B, Lee S-D *et al.* Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *J Gen Virol* 1993; 74: 1341–1348.

16 Arauz-Ruiz P, Norder H, Visoná KA, Magnius LO. Genotype F prevails in HBV infected patients of hispanic origin in Central America and may carry the precore stop mutant. *J Med Virol* 1997; 51: 305–312.

17 Rizzetto M. Viral hepatitis in the third millennium. *Res Virol* 1998; 149: 251–256.

18 Giusti G, Galanti B, Gaeta GB, Sagnelli E, Piccinino F, Ruggiero G. Clinical presentation and natural history of chronic persistent hepatitis. A multicentre retrospective study on 1197 cases. *Ital J Gastroenterol* 1991; 23: 111–118.

19 Matsuyama Y, Omata M, Yokosuka O, Imazeki F, Ito Y, Okuda K. Discordance of hepatitis B e antigen/antibody and hepatitis B virus deoxyribonucleic acid in serum. *Gastroenterology* 1985; 89: 1104–1108.

20 Shindo M, Hamada K, Koya S, Sokawa Y, Okuno T. The clinical significance of core promoter and precore mutations during the natural course and interferon therapy in patients with chronic hepatitis B. *Am J Gastroenterol* 1999; 94: 2237–2245.

21 Okamato H, Tsuda F, Akahane Y *et al.* Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994; 68: 8102–8110.

22 Takahashi K, Ohta Y, Kanai K *et al.* Clinical implications of mutations C-to-T^{1653} and T-to-C/A/G^{1753} of hepatitis B virus genotype C genome in chronic liver disease. *Arch Virol* 1999; 144: 1299–1308.

23 Lok ASF, Akarca U, Greene S. Mutations in the pre-core region of hepatitis B virus serve to enhance the stability of the secondary structure of the pre-genome encapsidation signal. *Proc Natl Acad Sci USA* 1994; 91: 4077–4081.

24 Stuyver L, De Gendt S, Van Geyt C *et al.* A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; 81: 67–74.

25 Rodriguez-Frias F, Buti M, Jardi R *et al.* Hepatitis B virus infection: Precore mutants and its relation to viral genotypes and core mutations. *Hepatology* 1995; 22: 1641–1647.

26 Chan HLY, Hussain M, Lok ASF. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. *Hepatology* 1999; 29: 976–984.

27 Brunetto MR, Giarin M, Saracco G *et al.* Hepatitis B virus unable to secrete e antigen and response to interferon in chronic hepatitis B. *Gastroenterology* 1993; 105: 845–850.

28 Fattovich G, Farci P, Rugge M *et al.* A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. *Hepatology* 1992; 15: 584–589.

29 Hadziyannis S, Bramou T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis type B. *J Hepatol* 1990; 11: S133–S136.

30 Pastore G, Santantonio T, Milella M *et al.* Anti-HBe-positive chronic hepatitis B with HBV DNA in the serum response to a 6-month course of lymphoblastoid interferon. *J Hepatol* 1992; 14: 221–225.

31 Zhang X, Zoulim F, Habersetzer F, Xiong S, Trépo C. Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. *J Med Virol* 1996; 48: 8–16.

32 Lok ASF, Akarca US, Greene S. Predictive value of precore hepatitis B virus mutations in spontaneous and interferon-induced hepatitis B e antigen clearance. *Hepatology* 1995; 21: 19–24.

33 Erhardt A, Reineke U, Blondin D *et al.* Mutations of the core promoter and response to interferon treatment in chronic replicative hepatitis B. *Hepatology* 2000; 31: 716–725.

34 Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2000; 32: 847–851.

35 Santantonio T, Mazzola M, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine. *J Hepatol* 2000; 32: 300–306.

36 Tassopoulos NC, Volpes R, Pastore G *et al.* Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. *Hepatology* 1999; 29: 889–896.

37 Tassopoulos NC, Volpes R, Pastore G *et al.* Post lamivudine treatment follow up of patients with HBeAg negative chronic hepatitis B. *J Hepatol* 1999; 30 (suppl 1): 117 (abstr).
38 Tassopoulos NC, Anagnostopoulos GD. Long-term lamivudine treatment of patients with precore mutant profile (HBeAg (−) /antiHBe (+)) chronic hepatitis B. *Antiviral Ther* 2000; 5 (suppl 1): 34 (abstract).
39 Wong FS, Fung S, Lok ASF, Hussain M. Use of lamivudine in Chinese patients with hepatitis B e-antigen negative chronic hepatitis B. *Hepatology* 2000; 32: A864.
40 Rizzetto M, Santantonio T, Buti M *et al.* Benefits of extended lamivudine treatment in patients with HBeAg-negative HBV DNA-positive (pre-core mutant) chronic hepatitis B (CHB). *Hepatology* 2000; 32: A1197.
41 Lok ASF, Hussain M, Cursano C *et al.* Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e-antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; 32: 1145–1153.
42 Nakahori S, Yokosuka O, Ehata T *et al.* Detection of hepatitis B virus precore stop codon mutants by selective amplification method: Frequent detection of precore mutants in hepatitis B e antigen positive healthy carriers. *J Gastroenterol Hepatol* 1995; 10: 419–425.
43 Niitsuma H, Ishii M, Saito Y *et al.* Prevalence of precore-defective mutant of hepatitis B virus in HBV carriers. *J Med Virol* 1995; 46: 397–402.
44 Ichikawa T, Takagi H, Kinoshita M, Shimoda R, Nagamine T, Mori M. Quantitative analysis of hepatitis B virus precore mutant in hepatitis type B. *Tohoku J Exp Med* 1998; 186: 323–333.
45 Nagasaka A, Hige S, Marutani M *et al.* Prevalence of mutations in core promoter/precore region in Japanese patients with chronic hepatitis B virus infection. *Dig Dis Sci* 1998; 43: 2473–2478.
46 Tian Q, Jia K. A kinetic study of hepatitis B virus pre-C gene mutation. *Chung Hua Nei Ko Tsa Chih* 1995; 34: 232–234.
47 Tu H, Li PY, Wen YM. Anti-HBe titre in patients infected with wild-type and e-minus variant of hepatitis B virus. *Res Virol* 1996; 147: 39–43.
48 Tu H, Xiong S-D, Trepo C, Wen Y-M. Frequency of hepatitis B virus e-minus mutants varies among patients from different areas of China. *J Med Virol* 1997; 51: 85–89.
49 Hou J, Lau GKK, Cheng J, Cheng CC, Luo K, Carman WF. T_{1762}/A_{1764} variants of the basal core promoter of hepatitis B virus; serological and clinical correlations in Chinese patients. *Liver* 1999; 19: 411–417.
50 Chu C-M, Karayiannis P, Fowler MJF, Monjardino J, Liaw Y-F, Thomas HC. Natural history of chronic hepatits B virus infection in Taiwan: Studies of hepatitis B virus DNA in serum. *Hepatology* 1985; 5: 431–434.
51 Wu J-C, Lee S-D, Tsay S *et al.* Symptomatic anti-HBe positive chronic hepatitis B in Taiwan with special reference to persistent HBV replication and HDV superinfection. *J Med Virol* 1988; 25: 141–148.
52 Liaw Y-F, Sheen I-S, Chen T-J, Chu C-M, Pao C-C. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: A prospective study. *Hepatology* 1991; 13: 627–631.
53 Chu C-M, Yeh C-T, Chiu C-T, Sheen I-S, Liaw Y-F. Precore mutant of hepatitis B virus prevails in acute and chronic infections in an area in which hepatitis B is endemic. *J Clin Microbiol* 1996; 34: 1815–1818.
54 Park YN, Han KH, Kim KS, Chung JP, Kim S, Park C. Cytoplasmic expression of hepatitis B core antigen in chronic hepatitis B virus infection: role of precore stop mutants. *Liver* 1999; 19: 199–205.
55 Kim WH, Kim KH, Chung JP, Kang JK, Park IS. Mutations in the pre-core region of hepatitis B virus DNA in patients with chronic liver diseases. *Yonsei Med J* 1993; 34: 158–165.
56 Sarin SK, Guptan RC, Banerjee K, Khandekar P. Low prevalence of hepatitis C viral infection in patients with non-alcoholic chronic liver disease in India. *J Assoc Physicians India* 1996; 44: 243–245.
57 Guptan RC, Thakur V, Sarin SK, Banerjee K, Khandekar P. Frequency and clinical profile of precore and surface hepatitis B mutants in Asian-Indian patients with chronic liver disease. *Am J Gastroenterol* 1996; 91: 1312–1317.
58 Yap I, Wee A, Guan R. Chronic hepatitis B infection in Singapore. *Singapore Med J* 1991; 32: 352–355.
59 Kidd-Ljunggren K, Ekdahl K, Öberg M, Kurathong S, Lolekha S. Hepatitis B virus strains in Thailand: Genomic variants in chronic carriers. *J Med Virol* 1995; 47: 454–461.
60 Ton SH, Iskandar K, Noriah R, Thanaletchimy N. Nucleotide sequence of precore region of hepatitis B virus DNA in HBsAg-positive carriers in Malaysia. *Scand J Infect Dis* 1996; 28: 543–548.
61 Realdi G, Alberti A, Rugge M *et al.* Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. *Gastroenterology* 1980; 79: 195–199.
62 Negro F, Chiaberge E, Oliviero S *et al.* Hepatitis B virus DNA (HBV-DNA) in anti-HBe positive sera. *Liver* 1984; 4: 177–183.
63 Alberti A, Pontisso P, Fattovich G *et al.* Changes in serum hepatitis B virus (HBV) DNA positivity in chronic HBV infection: Results of a long-term follow-up study of 138 patients. *J Infect Dis* 1986; 154: 562–569.
64 Bonino F, Rosina F, Rizzetto M *et al.* Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. *Gastroenterology* 1986; 90: 1268–1273.
65 Santantonio T, Jung M, Miska S, Pastore G, Pape G, Will H. High prevalence and heterogeneity of HBV preC mutants in anti-HBe-positive carriers with chronic liver disease in Southern Italy. *J Hepatol* 1991; 13 (suppl 4): S78–S81.
66 Brunetto MR, Giarin MM, Oliveri F *et al.* Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. *Proc Natl Acad Sci USA* 1991; 88: 4186–4190.
67 Lai ME, Solinas A, Mazzoleni AP *et al.* The role of pre-core hepatitis B virus mutants on the long-term outcome of chronic hepatitis B virus hepatitis. A longitudinal study. *J Hepatol* 1994; 20: 773–781.
68 Hadziyannis S, Lieberman H, Karvountzis G, Shafritz D. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg vs.

anti-HBe positive carriers of hepatitis B virus. *Hepatology* 1983; 3: 656–662.

69 Laras A, Koskinas J, Avgidis K, Hadziyannis SJ. Incidence and clinical significance of hepatitis B virus precore gene translation initiation mutations in e antigen-negative patients. *J Viral Hepat* 1998; 5: 241–248.

70 Jardi R, Buti M, Rodriguez-Frias F *et al.* The value of quantitative detection of HBV-DNA amplified by PCR in the study of hepatitis B infection. *J Hepatol* 1996; 24: 680–685.

71 López-Alcorocho JM, Moraleda G, Bartolomé J *et al.* Analysis of hepatitis B precore region in serum and liver of chronic hepatitis B virus carriers. *J Hepatol* 1994; 21: 353–360.

72 Tur-Kaspa R, Keshet E, Eliakim M, Shouval D. Detection and characterization of hepatitits B virus DNA in serum of HBe antigen-negative HBsAg carriers. *J Med Virol* 1984; 14: 17–26.

73 Tur-Kaspa R, Klein A, Aharonson S. Hepatitis B virus precore mutants are identical in carriers from various ethnic origins and are associated with a range of liver disease severity. *Hepatology* 1992; 16: 1338–1342.

74 Naoumov NV, Schneider R, Grötzinger T *et al.* Precore mutant hepatitis B virus infection and liver disease. *Gastroenterology* 1992; 102: 538–543.

75 Bozdayi A, Bozkaya H, Türkyilmaz A *et al.* Polymorphism of precore region of hepatitis B virus DNA among patients with chronic HBV infection in Turkey. *Infection* 1999; 27: 357–360.

76 Mangia A, Chung YH, Hoofnagle JH, Birkenmeyer L, Mushahwar I, Di Bisceglie AM. Pathogenesis of chronic liver disease in patients with chronic hepatitis B virus infection without serum HBeAg. *Dig Dis Sci* 1996; 41: 2447–2452.

77 Gray A, Fang J, Davis G *et al.* Variations of hepatitis B virus core gene sequence in Western patients with chronic hepatitis B virus infection. *J Viral Hepat* 1997; 4: 371–378.

78 Tillmann H, Trautwein C, Walker D *et al.* Clinical relevance of mutations in the precore genome of the hepatitis B virus. *Gut* 1995; 37: 568–573.

79 Knöll A, Rohrhofer A, Kochanowski B, Wurm EM, Jilg W. Prevalence of precore mutants in anti-HBe-positive hepatitis B virus carriers in Germany. *J Med Virol* 1999; 59: 14–18.

80 Zarski J, Marcellin P, Cohard M, Lutz J, Bouche C, Rais A. Comparison of anti-HBe-positive and HBe-antigen-positive chronic hepatitis B in France. French Multicentre Group. *J Hepatol* 1994; 20: 636–640.

81 Kidd Ljunggren K, Nordenfelt E, Kidd A. Correlation of HBeAg/Anti-HBe, ALT levels, and HBV DNA PCR results in HBsAg-positive patients. *J Med Virol* 1993; 39: 297–302.