Hepatitis B e Antigen–Negative Chronic Hepatitis B in Hong Kong

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Hepatitis B e antigen–negative chronic hepatitis B (e−CHB) has been reported in Asia but its prevalence and clinical significance have not been determined. The aims of this study were to determine the prevalence of e−CHB in Hong Kong and the frequency of precore and core promoter mutations in these patients. A cross-sectional study was performed in 350 consecutive Chinese patients (230 men and 120 women; mean age ±SD, 42 ± 13 years) with chronic hepatitis B virus infection. A total of 243 (69%) patients were hepatitis B e antigen (HBeAg)-negative of whom 15% had clinical cirrhosis. In the remaining 85% of patients, 63% had normal and 22% had elevated transaminases. Serum hepatitis B virus (HBV) DNA was detectable using branched DNA assay in 46% of HBeAg-negative patients with clinical cirrhosis/elevated transaminases. Forty-five percent of the patients with e−CHB had the precore stop codon mutation, and an additional 41% had core promoter changes. There was no correlation between the presence of precore/core promoter mutations and liver disease or HBV-DNA levels. Overall, 17% of HBeAg-negative patients were viremic and had evidence of chronic liver disease (e−CHB) with mean HBV-DNA levels comparable with that in HBeAg-positive patients. In summary, we found that e−CHB may be present in up to 17% of HBeAg-negative patients seen in a tertiary referral center in Hong Kong. e−CHB may be a heterogenous condition and is not invariably associated with the precore HBV mutant. Population studies are needed to determine the true prevalence of e−CHB in Asia and to assess its natural course and response to treatment. (HEPATOLOGY 2000;31:763-768.)

Seroconversion from hepatitis B e antigen (HBeAg) to e antibody (anti-HBe) is usually accompanied by cessation of hepatitis B virus (HBV) replication and remission of liver disease.[1,2] However, some HBeAg-negative patients remain viremic and continue to have active liver disease.[3-5] Many of these patients are found to have a G to A change at nucleotide 1896 (A1896), which creates a premature stop codon in the precore region of the HBV genome.[6-11] This mutation prevents the translation of the precore protein and completely abolishes the production of HBeAg. Other patients have mutations in the start codon of the precore region or mutations in the core promoter region.[9-15] The most common core promoter mutations involve a 2-nucleotide substitution: A to T at nucleotide 1762 and G to A at nucleotide 1764 (TA).[12-15] *In vitro* studies showed that the TA changes decrease transcription of precore messenger RNA and hence secretion of HBeAg.[16-19] Not all HBeAg-negative patients who are viremic have mutations that affect the secretion of HBeAg. Some continue to have low levels of wild type HBV.

The phenomenon of HBeAg-negative chronic hepatitis B (e−CHB) (hepatitis B surface antigen positive, HBeAg negative, serum HBV DNA detected by non–polymerase chain reaction [PCR] assay, and clinical/biochemical evidence of liver disease) was initially thought to be rare and largely confined to the Mediterranean basin. The prevalence of e−CHB is now considered to have surpassed that of e+CHB (hepatitis B surface antigen and HBeAg positive, serum HBV DNA detected by non-PCR assay, and clinical/biochemical evidence of liver disease) in some Mediterranean countries although population studies have not been performed to ascertain the relative frequency of e+ versus e−CHB. Several factors may account for the changing epidemiology. First, in the past decade, treatment of chronic hepatitis B has focused on HBeAg-positive patients because it was initially thought that HBeAg-negative patients have nonreplicative infection and do not require antiviral treatment. More recently, studies on interferon therapy in patients with e−CHB have reported high relapse rates discouraging treatment of these patients.[20-22] Second, transmission of HBV from patients with e−CHB may be facilitated by a lower level of vigilance in these patients[23] or a higher level of replication of the e− HBV mutant. *In vitro* studies suggest that mutations in the precore and/or core promoter region may increase the replication capacity of HBV[16,17,19] but clinical data comparing serum HBV-DNA levels in patients with and without these mutations are not available. Finally, it is possible that environmental changes in the last 1 to 2 decades may have favored the selection of the e− HBV mutants.

e−CHB has also been reported in the Middle East and in Asia such as in Japan[8,9,12,14] and Hong Kong[11,15] but is extremely rare in the United States.[24,25] The prevalence of e−CHB in different geographical regions is related to the predominant HBV genotype because the occurrence of A1896, the most common mutation associated with the HBeAg-negative phenotype, is restricted to HBV genotypes with T at

Abbreviations: HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBV, hepatitis B virus; A1896, G-A change at nucleotide position 1896 in the precore region; TA, A to T change at nucleotide 1762 and G to A change at nucleotide 1764 in the core promoter region; e−CHB, hepatitis B e antigen–negative chronic hepatitis B; PCR, polymerase chain reaction; T1858/C1858, T or C at nucleotide position 1858 in the precore region; ALT, alanine transaminase; bDNA, branched DNA.

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nucleotide 1858 (T1858).[11,24,26,27] In our previous studies in Hong Kong, we found that only 60% of Chinese patients who had chronic HBV infection were infected with HBV genotypes that have T1858[11,15] Of these, 87% to 100% had A1896 after HBeAg seroconversion. However, not all HBeAg-negative patients with A1896 had active liver disease. None of the patients infected with HBV genotypes that have C at nucleotide 1858 (C1858) developed A1896 after HBeAg seroconversion but 91% had TA changes in the core promoter region.[11,15]

With the availability of new therapies for chronic hepatitis B, knowledge of the prevalence of e−CHB may change our treatment strategies and focus research on the safety and efficacy of treatment for this expanding patient population. The aims of this study were to determine the prevalence of e−CHB in Hong Kong and the prevalence of precore and core promoter mutations among these patients.

PATIENTS AND METHODS

We performed a cross-sectional study in 350 consecutive Chinese patients with chronic HBV infection seen by a single hepatologist (N.W.-Y.L.) in the Hepatitis and General Hepatology Clinics, Prince of Wales Hospital, Hong Kong between December 1997 and June 1998. Patients who were coinfected with hepatitis C or D virus or human immunodeficiency virus and patients with other concomitant causes of chronic liver disease were excluded. The study population included 230 (66%) men and 120 (34%) women, aged 12 to 80 years (mean ± SD, 42 ± 13 years). Fifteen patients had previous antiviral treatment, 13 had interferon, 1 had famciclovir, and 1 had sequential treatments with interferon, lamivudine, and famciclovir. These patients were studied 1 to 120 months (median, 28 months) after the cessation of treatment. Patients who were actively participating in clinical trials on antiviral therapy of chronic hepatitis B were followed in a Research Clinic and were not included in this study. Less than 10% of patients had liver biopsies. The clinical status of the patients were stratified into three groups: Group I included noncirrhotic patients with normal alanine transaminase (ALT) levels, group II included noncirrhotic patients with elevated ALT levels (>upper limit of normal), and group III included patients with clinical evidence of cirrhosis. Among the 55 group III patients, all had ultrasound findings suggestive of cirrhosis (coarse liver architecture, nodular liver surface, and blunt liver edge) and evidence of hypersplenism (splenomegaly on ultrasound and platelet count <100,000/mm^3). In addition, 15 had esophageal varices on endoscopy and 4 had clinically detectable ascites.

All the patients were known to be HBsAg positive. All the patients were tested for HBeAg, anti-HBe, and liver profile (serum albumin, bilirubin, aspartate transaminase and ALT, and alkaline phosphatase levels) during the clinic visit. An additional sample of blood was drawn and tested for HBV DNA by the branched DNA (bDNA) assay as well as by an in-house PCR assay. Samples that were HBV-DNA positive by PCR were further studied to determine the core promoter and precore HBV sequence.

Hepatitis B serological markers were tested using commercially available enzyme-linked immunosorbent assay kits from Abbott Laboratories (North Chicago, IL). Serum HBV DNA was quantified using the bDNA assay (Quantiplex HBV DNA; Chiron Diagnostics, Emeryville, CA) which has a detection limit of 7×10^5 viral equivalents/mL.

PCR and direct sequencing of amplified products was performed as described previously using primers that flanked the core promoter and precore regions.[15] The sensitivity limit of the PCR assay was 500 viral equivalents/mL. This limit was determined by running duplicate dilution series of HBV plasmids with known concentrations and sera with known HBV-DNA levels based on the bDNA assay. All necessary precautions were taken to avoid cross-contamination during the PCR assay. Mutations were confirmed by bidirectional sequencing.

Two-tailed Student's *t* test was used to compare continuous variables. Two-tailed χ^2 test with Yates correction was used to compare categorical data. Mean HBV-DNA levels were compared after logarithmic transformation of the HBV-DNA values in the bDNA assay, and patients with undetectable HBV DNA in the bDNA assay were excluded.

RESULTS

Characteristics of the Patients Studied. Our study population included 107 (31%) HBeAg-positive and 243 (69%) HBeAg-negative patients. The HBeAg-negative patients were significantly older than the HBeAg-positive patients, mean ages ± SD for the HBeAg-negative and the HBeAg-positive patients being 45 ± 12 years and 36 ± 13 years, respectively ($P < .001$). The gender ratios in the HBeAg-positive (men/women, 68:39) and the HBeAg-negative (men/women, 162:81) patients were similar.

Clinical Status of the Patients. Fifty-five (16%) patients had clinical cirrhosis. Of the remainder, 194 (55%) had normal and 101 (29%) had elevated ALT levels. Patients with clinical cirrhosis were significantly older than those without clinical cirrhosis ($P < .001$) (Table 1). Among the noncirrhotic patients, the mean ages of those with normal (group I) and elevated (group II) ALT levels were similar.

Compared with HBeAg-positive patients, the HBeAg-negative patients were more likely to have normal ALT levels, 63% (153 of 243) versus 38% (41 of 107) ($P < .001$), but the proportions with clinical cirrhosis were similar, 15% (36 of 243) versus 18% (19 of 107) (Table 1).

Male patients were more likely to have elevated ALT levels, 34% (78 of 230) vs. 19% (23 of 120) ($P = .006$) and a higher tendency to have clinical cirrhosis, 18% (42 of 230) versus 11% (13 of 120).

HBV-DNA Detection in Relation to HBeAg Status and Liver Disease. Serum HBV DNA was detected in 39% (135 of 350) using the bDNA assay and in 64% (223 of 350) using the more sensitive PCR assay (Table 2). A significantly lower

TABLE 1. Clinical Status of Patients in Relation to HBeAg Status, Gender, and Age

Clinical Status	Total	No. (%) of Patients		Sex M/F	Mean Age ± SD (yr)
		HBeAg$^+$	HBeAg$^-$		
Noncirrhotic					
Normal ALT (group I)	194 (55)	41 (38)*	153 (63)*	110 (57)/84 (43)	41 ± 12†
Elevated ALT (group II)	101 (29)	47 (44)	54 (22)	78 (77)/23 (23)	40 ± 13†
Clinical cirrhosis (group III)	55 (16)	19 (18)	36 (15)	42 (76)/13 (24)	52 ± 14†
Total	350	107	243	230 (66)/120 (34)	42 ± 13

*ABeAg-positive vs. HBeAg-negative patients in group I ($P < .03$).

†Group I or II vs. group III, $P < .001$.

TABLE 2. HBV-DNA Detection in HBeAg-Positive and HBeAg-Negative Patients

HBeAg/ Clinical Status	No. of Patients	ALT (IU/L) Mean ± SD	Serum HBV DNA		
			No. (%) bDNA+	No. (%) PCR+	Log_{10} Mean SD (Eq/mL)
HBeAg positive					
Group I	41	39 ± 12	29 (71)*	35 (85)†	8.51 ± 1.30‡
Group II	47	168 ± 172	40 (85)§	42 (89)	7.93 ± 1.10
Group III	19	86 ± 79	14 (74)	18 (95)‖	7.26 ± 0.76
All	107	101 ± 130	83 (78)¶	95 (89)**	8.02 ± 1.20††
HBeAg positive					
Group I	153	34 ± 12	11 (7)*	64 (42)†	6.42 ± 0.38‡
Group II	54	152 ± 175	25 (46)§	40 (74)	7.37 ± 0.82
Group III	36	78 ± 52	16 (44)	24 (67)‖	6.86 ± 0.58
All	243	69 ± 117	52 (21)¶	128 (53)**	6.98 ± 0.78††

*$P < .001$.
†$P < .001$.
‡$P < .001$.
§$P < .001$.
‖$P < .05$.
¶$P < .001$.
**$P < .001$.
††$P < .001$.

percent of HBeAg-negative patients had detectable HBV DNA using the PCR assay: 53% (128 of 243) versus 89% (95 of 107) ($P < .001$) in HBeAg-positive patients. This difference was more marked when HBV DNA was detected using the bDNA assay: 21% (52 of 243) of HBeAg-negative versus 78% (83 of 107) of HBeAg-positive patients had detectable HBV DNA ($P < .001$) (Table 2). Among the patients who were bDNA positive, the HBeAg-negative patients had significantly lower HBV-DNA levels compared with the HBeAg-positive patients, log_{10} mean ± SD HBV-DNA levels being 6.98 ± 0.78 versus 8.02 ± 1.20 Eq/mL, respectively ($P < .001$) (Table 2, Fig. 1). The difference in HBV-DNA levels between the HBeAg-negative and HBeAg-positive patients was more marked in group I ($P < .001$) than in the group II patients ($P < .03$).

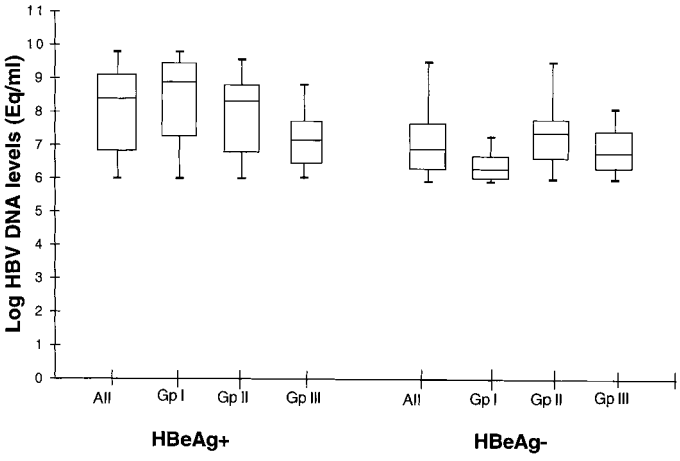

FIG. 1. Box-plot analysis of serum HBV-DNA levels in relation to HBeAg and clinical status. The boxes represent the second and third quartile and the "whiskers" extend from the lowest to the highest HBV-DNA levels in each group of patients. Gp I, noncirrhotic patients with normal ALT levels; GP II, noncirrhotic patients with elevated ALT levels; Gp III, patients with clinical cirrhosis.

Among the HBeAg-negative patients, HBV-DNA detection was strongly associated with the presence of liver disease (Table 2). Using the bDNA assay, HBV DNA was detected in the sera of only 7% (11 of 153) of patients in group I versus 46% (25 of 54) in group II ($P < .001$) and 44% (16 of 36) in group III ($P < .001$). In addition, group I patients had the lowest HBV-DNA levels (Table 2, Fig. 1). However, liver disease or ALT levels could not be predicted by the serum HBV-DNA levels because there was significant overlap among the three groups.

Contrary to the HBeAg-negative patients, HBV DNA was detected in similar proportions of groups I, II, and III HBeAg-positive patients. Moreover, HBeAg-positive group I patients had the highest HBV-DNA levels (Table 2, Fig. 1).

Prevalence of Core Promoter and Precore Stop Codon Mutations in Relation to HBeAg Status, HBV-DNA Detection, and Liver Disease. A1896 was mostly found in HBeAg-negative patients, 37% (47 of 128) versus 5% (5 of 95) in HBeAg-positive patients ($P < .001$), whereas TA changes were present in similar proportions of HBeAg-negative and HBeAg-positive patients, 65% (83 of 128) and 55% (52 of 95), respectively (Table 3).

Among the HBeAg-negative patients, 20% (26 of 128) had A1896 only, 48% (62 of 128) had TA changes only, and 16% (21 of 128) had both A1896 and TA changes (Table 3). The prevalence of A1896 and TA changes was similar in groups I, II, and III patients. HBV-DNA detection rates and mean HBV-DNA levels were comparable in HBeAg-negative patients with or patients without A1896, and in patients with or without TA changes.

Among the HBeAg-positive patients, there was a progressive increase in the prevalence of TA changes as the liver disease advances ($P = .003$). HBV-DNA detection rates were similar in HBeAg-positive patients with or without TA changes but HBV-DNA levels were significantly lower in patients with TA changes, log mean HBV-DNA levels being 7.30 ± 0.94 versus 8.88 ± 0.88, ($P < .001$).

HBV Genotypes in Relation to HBeAg Status and Core Promoter and Precore Stop Codon Mutations. Among the 223 patients with detectable HBV DNA by PCR, HBV genotypes with C1858 and T1858 were present in 118 (53%) and 105 (47%) patients, respectively. The distribution of HBV genotypes with C1858 and T1858 among the HBeAg-positive (57% vs. 43%) and the HBeAg-negative (50% vs. 50%) patients was similar. A1896 was exclusively found in patients with T1858, 73% (47 of 64) vs. 0% (0 of 64) HBeAg-negative patients with C1858 ($P < .001$) (Table 4). In contrast, TA changes could be

TABLE 3. HBV Core Promoter and Precore Mutations in Relation to HBeAg and Clinical Status

HBeAg/ Clinical Status	Total No. of Patients	No. (%) of Patients With			
		A1896 Only	A1896 + TA	TA Only	No Change
HBeAg positive					
Group I	35	2 (6)	0 (0)	9 (26)	24 (69)
Group II	42	1 (2)	1 (2)	26 (62)	14 (33)
Group III	18	0 (0)	1 (6)	15 (83)	2 (11)
All	95	3 (3)	2 (2)	50 (53)	40 (42)
HBeAg negative					
Group I	64	9 (14)	9 (14)	36 (56)	10 (16)
Group II	40	13 (33)	6 (15)	16 (40)	5 (13)
Group III	24	4 (17)	6 (25)	10 (42)	4 (17)
All	128	26 (20)	21 (16)	62 (48)	19 (15)

TABLE 4. HBV Core Promoter and Precore Mutations in Relation to HBV Genotype

Nucleotide at 1858	Total No. of Patients	No. (%) of Patients With			
		A1896 Only	A1895 + TA	TA Only	No Change
T1858	105	29 (28)	23 (22)	24 (22)	29 (28)
HBeAg$^+$	41	3 (7)	2 (5)	14 (34)	22 (54)
HBeAg$^-$	64	26 (40)	21 (33)	10 (16)	7 (11)
C1858	118	0	0	88 (75)	30 (25)
HBeAg$^+$	54	0	0	36 (67)	18 (33)
HBeAg$^-$	64	0	0	52 (81)	12 (19)

detected in patients with T or C at nucleotide 1858 and were more common among those with C1858, 81% (52 of 64) vs. 48% (31 of 64) of HBeAg-negative patients with T1858, ($P < .001$).

Other Mutations in the Core Promoter and Precore Regions. A variety of other mutations were found in the core promoter and precore regions (Table 5). The most common core promoter mutations included deletions (7-20 base pairs spanning nucleotide positions 1752-1777) in 5 patients, and a double nucleotide substitution of C with T and G with A at positions 1766 and 1768 in 16 patients. Of the latter 16 patients, all had concomitant T1762/A1764 changes, 10 were HBeAg negative, 7 (44%) had detectable HBV DNA using bDNA assay, and 7 had evidence of liver disease.

A precore start codon mutation was found in 9 patients, all of whom were HBeAg negative, all had evidence of liver disease, 7 (78%) had detectable HBV-DNA by bDNA assay. Other mutations in the precore region included a G to A change at nucleotide 1898 in 32 patients (14%), of whom 17 were HBeAg negative. All 32 patients had HBV genotypes with C1858, none had A1896, and 28 (88%) had TA changes. A1898 was more often found in patients with cirrhosis, 20% (11 of 55) vs. 7% (21/295) in group I and II patients ($P = .006$). Another common mutation involved a G to A change at nucleotide 1899 detected in 28 patients (13%), of whom 21 were HBeAg negative. Twenty-one patients had HBV genotypes with T1858, and 7 had C1858. Twelve patients had A1896 and 19 had TA changes. A1899 was also more common in patients with clinical cirrhosis: 24% (13 of 55) versus 5% (15 of 295) in groups I and II patients ($P < .001$).

DISCUSSION

In this study, we found that 69% of a consecutive series of 350 Chinese patients with chronic HBV infection seen by a single hepatologist in Hong Kong were HBeAg negative. This is higher than the 56% prevalence in a previously reported series of 224 patients in Hong Kong.[28] Several factors may have contributed to the difference in prevalence of HBeAg in these two series. First, patients in the current series were older, mean age 42 versus 35 years in the previous series. Second, the current study enrolled patients from the Hepatitis as well as Hepatology Clinics (where patients with clinical cirrhosis were seen), whereas the previous study enrolled patients from the Hepatitis Clinic only. Finally, although both studies excluded HBeAg-positive patients who were participating in clinical trials on antiviral therapy, it is possible that a higher percent of HBeAg-positive patients were enrolled into antiviral trials in 1998 than in 1987 when interferon was the only treatment being evaluated. However, review of the clinic records showed that only 4% of the patients seen in the Hepatitis and Hepatology Clinics at the Prince of Wales Hospital in the past 2 years were enrolled in antiviral trials. A population study is needed to determine if there is a genuine increase in the proportion of HBeAg-negative patients among patients with chronic HBV infection in Hong Kong.

Of the 243 HBeAg-negative patients, 37% had clinical/biochemical evidence of liver disease, of whom 46% had detectable serum HBV DNA using bDNA assay. Thus, overall, 17% of HBeAg-negative patients (or 12% of the entire cohort) fulfilled the definition of e−CHB (HBsAg positive, HBeAg negative, serum HBV DNA detected by non-PCR assay, and clinical/biochemical evidence of liver disease). However, this figure may be a gross underestimate of the true prevalence of e−CHB in Hong Kong because some HBeAg-negative patients may have fluctuating ALT levels and/or intermittent detection of serum HBV DNA using the bDNA assay, which has a detection limit of 7×10^5 viral equivalents/mL. In addition, some HBeAg negative patients may have active liver disease on histology despite normal ALT levels and undetectable serum HBV DNA especially when these tests were

TABLE 5. Other Mutations in the HBV Core Promoter and Precore Regions in Relation to HBeAg and Clinical Status

	No. of Patients	No. (%) of Patients With Core Promoter Mutations		No. (%) of Patients With Precore Mutations		
		Deletion	T1766/A1768	Start Codon	A1898	A1899
HBeAg positive						
Group I	35	0 (0)	2 (6)	0 (0)	4 (11)	2 (6)
Group II	42	1 (2)	1 (2)	0 (0)	6 (14)	1 (2)
Group III	18	0 (0)	3 (17)	0 (0)	5 (28)	4 (22)
All	95	1 (1)	6 (6)	0 (0)	15 (16)	7 (7)
HBeAg negative						
Group I	64	4 (6)	7 (11)	0 (0)	7 (11)	6 (9)
Group II	40	0 (0)	2 (5)	6 (15)	4 (10)	6 (15)
Group III	24	0 (0)	1 (4)	3 (13)	6 (25)	9 (38)
All	128	4 (3)	10 (8)	9 (7)	17 (13)	21 (16)
Total*	223	5 (2)	16 (7)†	9 (4)	32 (14)‡	28 (13)§

*Patients who were PCR positive.

†All 16 had concomitant T1762/A1764 changes.

‡All had C1858, 28 had concomitant T1762/A1764, none had A1896.

§7 had C1858, 21 had T1858, 19 had concomitant T1762/A1764 changes, 12 also had A1896.

assessed on one occasion only. We acknowledge that population studies are necessary to determine the true prevalence of e−CHB. Nevertheless, the results of this study indicate that e−CHB is not uncommon outside the Mediterranean basin. Because of the high HBV carrier rate in Asia, the absolute number of Asian patients with e−CHB may be significantly higher than that in the Mediterranean countries.

We found that only 37% of our HBeAg-negative patients had the precore stop codon mutation A1896 in contrast to patients in Italy where the vast majority of patients with e−CHB have A1896. The low prevalence of A1896 among Chinese patients is related to the codominance of HBV genotypes that have T or C at nucleotide 1858. In accordance with our previous studies, A1896 was not detected in any patient with HBV genotypes that have C1858. Even among patients with T1858, only 73% of our HBeAg-negative patients had A1896 underscoring the fact that e−CHB may be a heterogenous condition and is not invariably associated with precore HBV mutant. Approximately two thirds of our HBeAg-negative patients had core promoter mutations (T1762/A1764) that had been shown to decrease HBeAg production. As in our previous report,[15] the TA changes were more often found in patients with HBV genotypes that have C1858, which preclude the development of the precore stop codon mutation. The finding of a high prevalence of TA changes in HBeAg-positive patients is not surprising because we have previously shown that these changes may precede HBeAg seroconversion by 5 years.

Several studies reported an association between severity of liver disease and the common core promoter (T1762/A1764) and precore (A1896) mutations.[7,9,29-31] More recently, there have also been reports incriminating the T1762/A1764 mutations in hepatocarcinogenesis.[32,33] In this study, the prevalence of A1896 and T1762/A1764 changes was similar in the three groups of HBeAg-negative patients confirming our previous reports[15] of a lack of correlation between these mutations and liver disease. The high prevalence (61%) of T1762/A1764 changes in our patients, none of whom had hepatocellular carcinoma, and our previous observation of patients who remained tumor-free up to 7 years after the detection of these changes[15] do not support a role of T1762/A1764 core promoter mutations in hepatocarcinogenesis. Another double mutation (T1766/A1768) in the core promoter region had been previously reported in a patient with fulminant hepatitis.[34] These changes were detected in 7% of our patients and were found in all three groups of patients. We found that the only mutations that appeared to be associated with more severe liver disease involved codon 29 in the precore region (G to A, nucleotide 1898 and G to A, nucleotide 1899). Other investigators have also reported an association between severity of liver disease and A1899.[7,35] *In vitro* studies suggested that precore and core promoter mutations may enhance HBV replication thus accounting for increased pathogenicity.[16,17,19] We found no difference in the rate of HBV-DNA detection or HBV-DNA levels among HBeAg-negative patients with or without precore stop codon mutation. Although a higher percent of HBeAg-negative patients with TA changes had detectable serum HBV DNA, the HBV-DNA levels were similar in patients with and without core promoter changes.

As a group, the HBeAg-negative patients were less likely to have detectable serum HBV DNA especially when tested by a non-PCR based assay and had lower HBV-DNA levels. These findings were true for the vast majority of group I patients who had normal ALT levels. In contrast, group I HBeAg-positive patients had the highest HBV-DNA levels. Thus, the clinical significance of HBsAg-positive carriers with normal ALT levels may be very different depending on the HBeAg status. Unlike patients with normal ALT levels, 46% of our HBeAg negative patients who had elevated ALT levels or clinical cirrhosis had detectable serum HBV DNA by bDNA assay with levels that were comparable with those in the HBeAg-positive patients suggesting that antiviral therapy may be beneficial in these patients. Interferon has been shown to be effective in suppressing HBV replication and in normalizing ALT levels in patients with e−CHB but relapse is common after cessation of treatment.[20-22] Recently, lamivudine has been reported to be effective in inducing biochemical, virological, and histological response in anti-HBe–positive patients with chronic hepatitis.[36] Most of the patients in that study were Italians and Greeks. Whether these encouraging results can be applied to Asian patients and whether the response can be maintained after withdrawal of lamivudine remain to be determined.

In summary, we found that e−CHB may be present in up to 20% of HBeAg-negative patients seen in a tertiary referral center in Hong Kong. These patients had HBV-DNA levels that were comparable with HBeAg-positive patients suggesting that they may benefit from antiviral therapy. Contrary to e−CHB seen in Mediterranean countries, only 37% of our patients had the precore stop codon mutant. Thus, it is unclear if Asian patients with e−CHB have the same natural course and response to treatment as the Italian and Greek patients. Given the high HBV carrier rate in Asia, population studies to determine the true prevalence of e−CHB in Asia and its natural course and response to treatment are urgently needed.

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