CHRONIC HEPATITIS B INFECTION IN SINGAPORE

I Yap, A Wee, R Guan

ABSTRACT

Four hundred and four patients (273 men, 131 women) aged 3 to 85 years with chronic Hepatitis B virus (HBV) infection seen during a five year period were analysed. At presentation, 177 patients (44%) were Hepatitis B e Antigen (HBeAg) positive (mean age 32 years) and 217 patients (54%) were anti-HBe-positive (mean age 40 years). Ten patients (2%) were negative for HBeAg and anti-HBe. Serum HBV-DNA was detected in 169 patients (42%). 85% of the HBeAg-positive patients had detectable serum HBV-DNA and 9% of the HBeAg-negative patients were positive for serum HBV-DNA. The mean serum Alanine amino-transferase (ALT) and Aspartate amino-transferase (AST) levels were higher in HBeAg-positive patients (75 and 52 iu/l) than in HBeAg negative patients (46 and 37 iu/l) (P<0.001). Liver biopsies were performed in 135 patients. Fifty-three (39%) had minimal changes, 61 (45%) chronic hepatitis (CPH, CLH & CAH) and 21 (16%) cirrhosis. There was no significant difference in the histologic distribution between HBeAg-positive and HBeAg-negative groups. Two hundred and fifty eight patients were followed up for a mean duration of 2 years (range 3 to 108 months). The cumulative probability of clearing HBeAg at the end of the first, second and third year were 14%, 16% and 18% respectively. Of these, the cumulative probability of developing anti-HBe over one, two and three years were 8%, 9% and 11% respectively. Reversion to HBeAg occurred in 1.5% of patients who were HBeAg-negative at presentation and 11% of HBeAg-positive patients who cleared HBeAg. Four patients (1.5%) lost Hepatitis B s Antigen (HBsAg) and developed anti-HBs within 5 to 30 months (mean 15 months) of follow up.

Keywords: Hepatitis B s antigen, Hepatitis B e antigen, HBV-DNA, chronic hepatitis B virus infection, spontaneous seroconversion.

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INTRODUCTION

Chronic HBV infection occurs when HBsAg remains in the serum for more than 6 months. The serum HBeAg is a protein which is similar in structure to the Hepatitis B core antigen (HBcAg) and is a practical marker of viral replication and therefore infectivity in HBV infection. This antigen however does not persist indefinitely as spontaneous seroconversion from HBeAg to anti-HBe can occur during the course of chronic HBV infection. Infectivity decreases when HBeAg disappears from the serum but not invariably so as some of these HBeAg-negative individuals still have HBV-DNA (a more sensitive indicator of infectivity) in their sera. Knowledge of the frequency of HBeAg positivity and the seroconversion rate of carriers in a particular region are essential in the management and evaluation of treatment regimes for chronic HBV infection. We analysed 404 chronic HBV carriers' serologic, biochemical and histologic parameters during a 5-year follow up period in our department to determine a) the change of these parameters with the natural evolution of the disease and b) the rate of spontaneous HBeAg to anti-HBe seroconversion and reversion.

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MATERIAL AND METHODS

Patients

All chronic hepatitis B carriers seen at the Department of Medicine, National University Hospital by two of the authors (IY & RG) during a five year period were analysed. Patients who had immunosuppressive or anti-viral therapy during the study period and those who had histologically proven hepatocellular carcinoma were excluded.

Carriers were seen at 3 to 6 months intervals or more frequently when clinically indicated. At each visit, the patients were assessed clinically and blood was taken for liver function tests, alpha-fetoprotein (AFP) and HB serology. Percutaneous needle liver biopsies were performed (if ALT>55 u/l or abnormal liver ultrasonography) at the time of presentation or shortly afterwards.

Serological Assays

Serum samples were tested for HBsAg, HBeAg and anti-HBe by commercially available enzyme linked immunoassay kits (Auszyme II for HBsAg, EIA for HBeAg/AntiHBe, Abbott Laboratories, North Chicago 111). Serum HBV deoxyribonucleic acid (HBV-DNA) was measured by molecular hybridization using a ^{32}P-labelled HBV-DNA probe described previously[1]. The hybridization reaction when present (positive) was semi-quantitatively graded on the basis of the spot intensity on the resultant autoradiograph[2].

Liver biopsy specimens

Formalin-fixed liver biopsy specimens were processed routinely. Paraffin-embedded sections were stained with haematoxylin and eosin, Masson's trichrome, Gordon and Sweets' method for reticulin, Perls' prussian blue method for iron and periodic acid-Schiff technique with and without diastase digestion. The specimens were classified according to standard histological criteria with slight modifications[3]. The groups were: minimal changes, chronic persistent hepatitis (CPH), chronic lobular hepatitis (CLH), chronic active hepatitis (CAH) and cirrhosis. For this study, three main categories were used, viz. minimal changes, chronic hepatitis (comprising CPH, CLH, CAH) and cirrhosis.

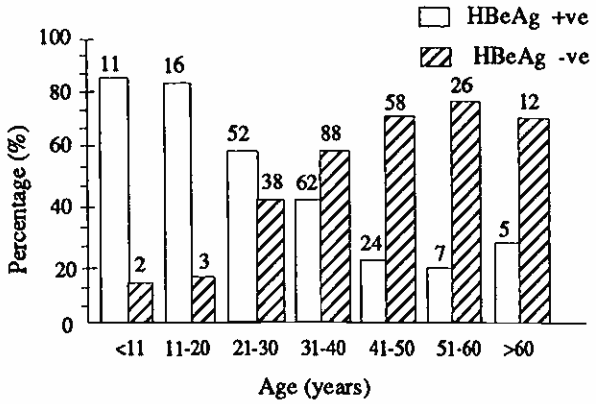
Statistical Analysis

Categorical data were compared using Fisher's exact probability test and p values < 0.05 were taken to be significant.

RESULTS

There were 404 patients consisting of 273 men (68%) and 131 women (32%). Their ages ranged from 3 to 85 years (mean age 36 years). The number of patients in each age group is as shown in Fig 1.

Fig 1 – Frequency of HBeAg-positive and HBeAg-negative status according to age groups



I) At presentation:

Serological characteristics

There were 177 (44%) HBeAg-positive and 227 (56%) HBeAg-negative carriers. Of the HBeAg-negative patients, 96% (217/227) were anti-HBe positive and 4% (10/227) anti-HBe negative. The mean age of HBeAg-positive carriers was 32 years while that of HBeAg-negative carriers was 40 years ($p<0.001$). Serum HBV-DNA was detected in 169 patients (42%). 85% (148/175) of the HBeAg-positive patients had detectable serum HBV-DNA and 9% (21/227) of the HBeAg-negative patients were positive for serum HBV-DNA (Table I).

Table I
Correlation of serum HBeAg/anti-HBe with HBV-DNA status in chronic HBV carriers at presentation

	No.	HBV-DNA +ve	HBV-DNA-ve
HBeAg +ve	175*	148/175	27/175
anti-HBe -ve		(85%)	(15%)
HBeAg -ve	10	1/10	9/10
anti-HBe -ve		(10%)	(90%)
HBeAg -ve	217	20/217	197/217
anti-HBe +ve		(9%)	(91%)
Total	402	402	402

* 2 patients had no HBV-DNA measured

Biochemical characteristics

The mean ALT and AST levels of HBeAg-positive carriers at presentation were significantly higher than those of HBeAg-negative carriers: viz 75 and 52 iu/l vs 46 and 37 iu/l respectively ($p<0.001$).

Histologic characteristics

Liver biopsies were performed on 135 patients. Thirty-nine percent (53) had minimal changes, 45% (61) chronic hepatitis (CPH, CLH, CAH) and 16% (21) cirrhosis. There was no significant difference in the distribution of histologic changes between the HBeAg-positive and HBeAg-negative groups (Table II).

II) Follow up:

Two hundred and fifty-eight patients were followed up for a mean duration of 2 years (ranging from 3 to 108 months). There were 101 HBeAg-positive, 147 HBe-negative/anti-HBe-positive and 10 HBeAg/anti-HBe-negative carriers. The rest were either seen only once or were selected for interferon trial therapy.

Table II
Histologic distribution in chronic HBV carriers

	No.	HBeAg +ve	HBeAg -ve
Minimal changes	53 (39%)	31 (36%)[a]	22 (46%)[b]
Chronic hepatitis (CPH, CLH, CAH)	61 (45%)	44 (50%)[c]	17 (35%)[d]
Cirrhosis	21 (16%)	12 (14%)[e]	9 (19%)[f]
Total	135 (100%)	87 (64%)	48 (36%)

$p^{a \text{ vs } b} = NS$
$p^{c \text{ vs } d} = NS$
$p^{e \text{ vs } f} = NS$

HBeAg-positive carriers

One hundred and one patients (65 men and 36 women) were followed up for 3 to 72 months. Eighteen (12 men and 6 women) became seronegative for HBeAg during the study period. The majority cleared their HBeAg within a year of presentation. Their mean age was 32 years and they had a mean ALT level of 76 iu/l at presentation. The cumulative probability of clearing HBeAg at the end of the first, second and third year of follow up were 14%, 16% and 18% respectively.

Eleven of these patients who became seronegative (7 men and 4 women) seroconverted to anti-HBe-positive within 2 to 30 months. The cumulative probability of developing anti-HBe over one, two and three years were 8%, 9% and 11% respectively. Two patients (11%), both men, reverted to HBeAg-positive state within a year. The remaining 5 (3 men and 2 women) remained HBeAg- and anti-HBe-negative (window period) during the course of the study period.

HBeAg-negative carriers

Ten patients (all men) had no 'e' markers at the time of presentation. Their mean age was 34 years. Eight converted to anti-HBe-positive state within 3 to 42 months (mean 16 months) whilst 2 remained without 'e' markers throughout the follow up period.

Among the 147 anti-HBe-positive patients (103 men, 44 women), three of them (2 men and one woman) reverted to HBeAg-positive state within 3 to 7 months (mean 5 months).

Four patients (3 men and one woman) lost their HBsAg and developed anti-HBs within 5 to 30 months (mean 15 months). Their mean age was 60 years. HBsAg became undetectable in one 62-year-old man with cirrhosis 30 months after presentation. He later developed anti-HBs. A 72-year-old woman lost her HBsAg after 5 months of follow up. The third patient, a 55-year-old man presented with hepatomegaly and was found to be HBsAg positive. Ultrasonography showed a small intrahepatic nodule but fine needle aspiration of this nodule did not reveal any evidence of malignancy. He seroconverted to anti-HBs after 7 months of follow up. A 52-year-old man was found to be HBsAg positive on routine testing 2 years prior to being seen. A repeat HBsAg at our clinic was negative, anti-HBe, anti-HBc and anti-HBs were present in his serum.

Correlation between 'e' seroconversion/reversion and serum HBV-DNA levels (Table III)

Serum HBV-DNA became undetectable before or soon after the HBeAg disappeared in all patients who cleared their HBeAg during follow up. Of the three patients who reverted from anti-HBe to HBeAg-positive state during follow up, the serum HBV-DNA became positive in 2 of them during 'e' reversion.

Only one of the 4 patients who seroconverted to anti-HBs positive had serum HBV-DNA at presentation and HBV-DNA

Table III
Characteristics of chronic HBV carriers who clear vs those who do not clear serum HBeAg during the study period

	Cleared HBeAg	Remaining HBeAg +ve
Number	18	83
Men : Women	12 : 6 (2 : 1)	52 : 31 (1.7 : 1)
Mean age (years)	32	32
Mean ALT level at presentation (iu/l)	81[a]	74[a]
HBV-DNA status at presentation		
+ve	8/17 (47%)[b]	75/81 (93%)[b]
-ve	9/17 (53%)[c]	6/81 (7%)[c]

p[a] = NS
p[b] 0.0001
p[c] 0.0001

disappeared soon after 's' seroconversion after 2.5 years of follow up. They were all HBeAg negative and anti-HBe positive presentation.

Of the 83 patients who remained HBeAg positive during follow up, serum HBV-DNA was present persistently in most of them (72). Serum HBV-DNA became negative in 6 while 5 others had fluctuating levels: positive at times and undetectable at others.

Twenty HBeAg-negative patients had serum HBV-DNA when first seen. HBV-DNA became undetectable in 10 of them on follow up in spite of seroreversion to HBeAg-positive in one of them. HBV-DNA remained positive in 4 of them during the study period. Six others failed to come back for review.

DISCUSSION

The prevalence of serum HBeAg in asymptomatic HBV carriers varies considerably in different parts of the world. Childhood infection is common in Asia and this early infection could be responsible for higher rates of chronic HBV carriage as well as a prolonged and greater HBeAg prevalence[4-7]. A high proportion (44%) of our patients had 'e' antigenaemia. This trend is also found in other parts of Asia[8,9].

There was a progressive decrease in the frequency of HBeAg positivity with age indicating a reduction in virus replication with time. The HBeAg/anti-HBe status generally correlates well with the presence or absence of serum HBV-DNA; the latter being a more sensitive marker of viral replication. Up to 10% of our patients who are anti-HBe positive still have evidence of virus replication. A recent study showed that mutations in the terminal two codons of the pre-core region is probably the cause of the absence of 'e' antigen in these patients[10]. Persistence of HBV replication is important in disease progression. Viral replication persisting after anti-HBe seroconversion is thought to result in more liver damage as the abnormal translated protein resulting from the pre-core mutation could be cytotoxic. The development of cirrhosis and hepatocellular carcinoma is therefore more likely in these patients.

The decrease in frequency of HBeAg accompanied by a reciprocal increase of anti-HBe with age indicate that seroconversion of HBeAg to anti-HBe occurs spontaneously in chronic HBV infection. Seroconversion is usually preceded by an acute hepatitic event followed by improvement of serum transaminase levels and a reduction in HBV replication[11]. The mechanism of this 'e' antigen clearance is probably immunological. Hoofnagle et al postulated that during the HBeAg-positive phase, active viral replication may have resulted in some virus-induced antigen (probably hepatitis B core antigen) being manifested on the surface of hepatocytes harbouring the complete virion. These hepatocytes are subsequently recognised by the host immune system with resultant destruction[12]. After destruction of the hepatocytes which actively produce HBV, there is no other source of HBeAg production and only its antibody remains. However, not all exacerbations occurring in HBeAg-positive patients lead to loss of HBeAg as some make many seroconversion. Reversion occurs as well after apparent clearance of HBeAg; this being frequently observed in those patients without simultaneous development of anti-HBe[9]. Clearance of HBeAg was associated with simultaneous detection of anti-HBe in 61% (11/18) of our patients. 11% (2/18) reverted within a year of clearance of HBeAg. Because of the relatively long intervals between follow up visits, acute exacerbations and transient reversions may have been missed.

Seroconversion starts to increase from 21-30 years of age and become maximal between 51-60 years of age locally (Fig 1). This observation differs from other reports in which seroconversion seems to occur more frequently in the teens and early adulthood[13] and could be due to small number of patients in the below 20 age group in our study. Factors predicting anti-HBe seroconversion have been searched for but with disappointing results so far[14,15]. We found a significantly lower HBV-DNA positivity at presentation among those who cleared their serum HBeAg subsequently as compared with those who did not clear. However, there is no difference in sex distribution, mean age and mean ALT level at presentation among the two groups (Table III).

Spontaneous reactivation of the virus resulting in disease activity may also occur in previously asymptomatic anti-HBe-positive carriers and the prognosis in these patients seems poor[16]. In the 3 patients who reverted from anti-HBe to HBeAg-positive state, the serum HBV-DNA became detectable at the time of HBeAg reversion. This trend was also observed in other studies[4,5].

Equal numbers of chronic hepatitis and cirrhosis were seen in both HBeAg-positive and HBeAg-negative patients locally. An Italian study showed similar trends[17]. Other workers found a low prevalence of HBeAg and a higher prevalence of anti-HBe in cirrhosis[18-20]. Late 'e' seroconversion and the presence of HBV-DNA in anti-HBe patients in the local population may explain this difference in findings.

Seroconversion from HBsAg to anti-HBs is a rare event[21]. With anti-viral therapy, a small proportion of carriers may clear their HBsAg[22]. Our study showed that 's' seroconversion occurs at a relatively old age. One of our patients underwent 's' seroconversion after he developed cirrhosis. This 's' seroconversion could be responsible for some cases of non-B, non-alcohol related cirrhosis in this part of the world.

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