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INCIDENCES OF HBeAg AND ANTI-HBe IN, AND CLINICAL COURSE OF HEPATITIS B VIRUS CARRIERS

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SUMMARY A total of 336 hepatitis B virus (HBV) carriers were followed for more than 3 years with serial measurements of serological markers of HBV to determine the correlation between their clinical course and the HBeAg-anti-HBe system. In all, 139 had hepatitis B e antigen (HBeAg) at the beginning of the study. During the study, 30 of 139 HBeAg-positive carriers became HBeAg negative and subsequently gave a positive reaction for antibody to HBeAg (anti-HBe). The rate of seroconversion was 3.6% per year. Two types of profile of seroconversion were observed, rapid and gradual. No significant differences were observed in the incidences of HBeAg and anti-HBe in HBV carriers with or without liver cirrhosis (LC) and hepatocellular carcinoma (HCC). These findings do not support the report by Chung et al. (1983, J. Med. Virol. 11: 99–104) that a prolonged replicative phase of chronic HBV infection is essential for the occurrence of HCC. Two HBV carriers were diagnosed as having HCC at the time of seroconversion from HBeAg to anti-HBe. This finding supports the reports by Coursaget et al. (1978, J. Clin. Microbiol. 7: 394–395) and Musca et al. (1983, Hepatogastroenterology 30: 3–5) that actively replicating HBV sometimes becomes defective during the course of malignant transformation.

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is very high in China, Southeast Asia, Japan, and Africa, whereas its incidence is relatively low in the USA and Western Europe (Szmuness, 1978). Hepatitis B virus (HBV) infection is common in areas of the world where HCC is common. Thus, epidemiological studies have shown a very close correlation between the geographical frequency of the HBV carrier state and the incidence of

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HCC. Furthermore, a higher frequency of hepatitis B surface antigen (HBsAg) was found in the serum of patients with HCC than in that of controls (Coursaget et al., 1981; Chung et al., 1983). Recently, an HCC cell line was shown to produce HBsAg, and HBV-DNA was found to be integrated into the genome of these cells (Brechot et al., 1980; Marion et al., 1980). These findings strengthen the idea that HBV plays a major role in the malignant transformation of liver cells. Chung et al. (1983) reported that predisposition of a prolonged replicative phase of chronic HBV infection is essential for the occurrence of HCC. In this study, we examined the incidences of hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) in HBV carriers with or without liver cirrhosis (LC) and HCC to determine the correlation between the HBeAg-anti-HBe system and the clinical course of HBV carriers. This study is on greater numbers of subjects with more thorough follow-up of HBV carriers than studies performed by others.

MATERIALS AND METHODS

In a serologic survey for HBV infection in patients in Kure National Hospital between February 1970 and January 1983, 1,649 individuals had HBsAg in their serum. All these patients were advised to return to hospital for follow-ups every 6 months. Routine follow-up studies included clinical assessment, conventional liver function tests, and tests for serological HBV markers.

1. *Follow-up study on the HBeAg-anti-HBe system in HBV carriers*

Among 1,649 individuals, 336 could be followed regularly for over 36 months (mean, 71 months), to examine the long-term course of change in the HBeAg-anti-HBe system. The 336 HBV carriers consisted of 181 men and 155 women of 2 to 86 years old (mean, 37 years).

2. *Incidences of HBeAg and anti-HBe among HBV carriers without LC or HCC*

From January 1981 to December 1982, sera were obtained from 621 HBV carriers. This study group included 18 LC patients and 4 HCC patients and 10 carriers who exhibited change in the HBeAg-anti-HBe system during the period. Of the 621 carriers, 589 individuals, excluding these 32, were examined for HBeAg and anti-HBe. They consisted of 313 males and 276 females, ranging from 6 to 85 years old. These 589 subjects included 298 of the 336 individuals who were followed up.

3. *Prevalences of HBeAg and anti-HBe among HBV carriers with LC and HCC*

For examination of the incidences of HBeAg and anti-HBe in the sera of patients with LC and HCC, we obtained 44 sera of HBV carriers with LC and 29 sera of HBV carriers with HCC. The 44 patients with LC consisted of 36 men and 8 women of 19 to 85 years old (mean, 53 years), while the 29 patients with HCC consisted of 24 men and 5 women of 32 to 71 years old (mean, 57 years). Diagnosis of LC was based on histological or scintigraphic findings on the liver and results of liver function tests, and that of HCC was based on histologic or clinical findings on the liver by examinations such as angiography, liver scintigraphy and abdominal CT scanning in addition tests indicating elevation of the serum alpha fetoprotein level.

4. *Serological studies*

HBsAg was assayed by reversed passive hemagglutination and by radioimmunoassay (Ausria II, Abbott Laboratories, North Chicago, Ill). Antibody to HBsAg (anti-HBs) was assayed by a test for passive hemagglutination and by radioimmunoassay (Ausab, Abbott Laboratories, North Chicago, Ill). HBeAg and anti-HBe were measured by enzyme-linked immunosorbant assay (Eiken Immunochemical Laboratories, Tokyo, Japan) (Itoh et al., 1983) and radioimmunoassay (Abbott-HBe, Abbott Laboratories, North Chicago, Ill). Preliminary studies showed that enzyme-linked immunosorbant assay and radioimmunoassay had similar sensitivities for detections of HBeAg and anti-HBe in serum.

5. *Statistical analysis*

The incidences of HBeAg and anti-HBe were compared by the Chi-square test.

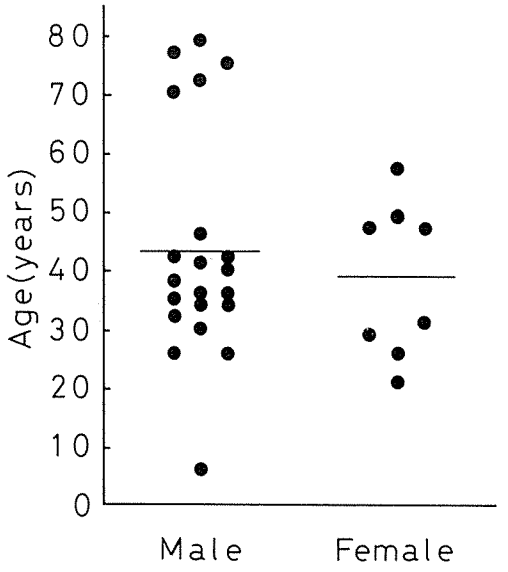

FIGURE 1. Age distribution of the time of seroconversion from HBeAg to anti-HBe. The time of seroconversion is shown as the age when HBeAg became negative and anti-HBe positive. Horizontal bars indicate mean ages.

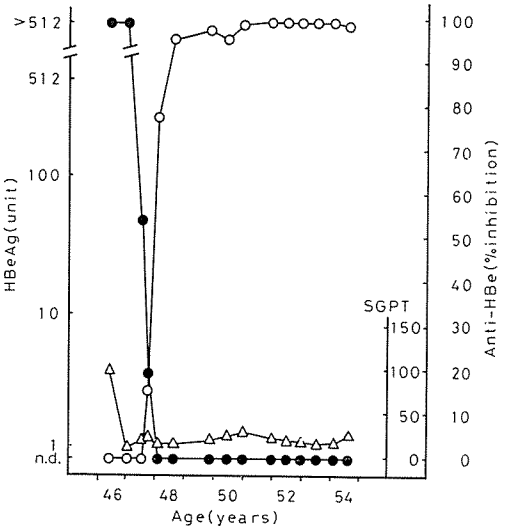

FIGURE 2. Course of seroconversion from HBeAg to anti-HBe (rapid seroconversion type). One unit of HBeAg was defined as the concentration of HBeAg at the highest dilution of the positive reference serum in which the antigen could be detected. Symbols: ●, HBeAg; ○, anti-HBe; △, serum glutamic-pyruvic transaminase (SGPT).

RESULTS

1. *Follow-up study on the HBeAg-anti-HBe system in HBV carriers*

Among 336 HBV carriers followed up for more than 36 months, 2 (0.6%) became HBsAg negative. Of the 336 individuals, 139 (41.4%) were positive for HBeAg at the beginning of this study. These 139 HBV carriers consisted of 89 men and 50 women of 5 to 86 years old (mean, 36 years), and their follow-up period ranged from 36 to 117 months (mean, 72 months). Of the 139 HBeAg-positive individuals, 30 (21.6%) became HBeAg negative and subsequently anti-HBe positive. The rate of seroconversion was 3.6% per year, 4.1% in 22 males and 2.7% in 8 females. As shown in Fig. 1, the age distribution of the time of seroconversion varied widely from 6 to 79 years (mean, 42 years). Two types of course of seroconversion were observed; in one type seroconversion occurred

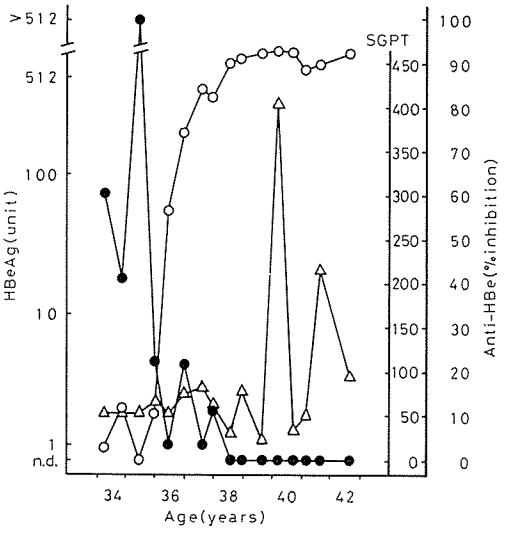

FIGURE 3. Course of seroconversion from HBeAg to anti-HBe (gradual seroconversion type). Explanations and symbols are as for Fig. 2.

rapidly as soon as HBeAg began to decrease quickly (Fig. 2) and in the other type it occurred slowly with a fairly long period of coexistence of both HBeAg and anti-HBe (Fig. 3). There was no correlation between the age and the type of course of seroconversion.

Of the 336 HBV carriers followed for over 36 months, 2 (0.6%) developed HCC and subsequently died. Both of these were males, and their ages when HCC was diagnosed were 32 and 43 years respectively. Initially they had HBeAg in the serum and seroconversion to anti-HBe-positive was detected when HCC was diagnosed.

2. *Prevalence of HBeAg and anti-HBe among HBV carriers without LC or HCC*

The distributions of HBeAg and anti-HBe in the sera of 589 HBV carriers obtained between January 1981 and December 1982 classified according to age groups are summarized in Table 1. Data on 18 HBV carriers with LC, 4 with HCC and 10 carriers, who exhibited changes in the HBeAg-anti-HBe system during this period are excluded from Table 1. Of 589 HBV carriers, 139 (23.6%) had only HBeAg and 417 (70.8%) had only anti-HBe, while 9 (1.5%) had both HBeAg and anti-HBe and 24 (4.1%) had no detectable HBeAg or anti-HBe. There was no significant difference between males and females in the incidences of HBeAg and anti-HBe in different age groups. The incidence of HBeAg was about 60% in groups of less than 20 years old, and decreased in older subjects, being about 20% in groups of over 30 years old. The incidence of anti-HBe was

TABLE 1. *Incidences of HBeAg and anti-HBe among HBV carriers according to age*

Age (years)	No. of subjects	HBeAg (+) anti-HBe (−)	HBeAg (−) anti-HBe (+)	HBeAg (+) anti-HBe (+)	HBeAg (−) anti-HBe (−)
0– 9	8	5(62.5)[a]	3(37.5)	0(0)	0(0)
10–19	24	16(66.7)	6(25.0)	0(0)	2(8.3)
20–29	87	31(35.6)	54(62.1)	0(0)	2(2.3)
30–39	185	35(18.9)	140(75.7)	4(2.2)	6(3.2)
40–49	139	22(15.8)	102(73.4)	3(2.2)	12(8.6)
50–59	70	10(14.3)	58(82.9)	1(1.4)	1(1.4)
60–69	49	11(22.4)	37(75.5)	1(2.0)	0(0)
70–	27	9(33.3)	17(63.0)	0(0)	1(3.7)
Total	589	139(23.6)	417(70.8)	9(1.5)	24(4.1)

Abbreviations: HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg; HBV, hepatitis B virus.
[a] Figures in parentheses are percentages.

TABLE 2. *Incidences of HBeAg and anti-HBe in HBV carriers with LC and HCC*

Diagnosis	No. of subjects	HBeAg (+) anti-HBe (−)	HBeAg (−) anti-HBe (+)	HBeAg (+) anti-HBe (+)	HBeAg (−) anti-HBe (−)
LC	44	12(27.3)[a]	27(61.4)	0(0)	5(11.4)
HCC	29	7(24.1)	18(62.1)	2(6.9)	2(6.9)

Abbreviations: HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg; HBV, hepatitis B virus; LC, liver cirrhosis; HCC, hepatocellular carcinoma.
[a] Figures in parentheses are percentages.

about 30% in subjects of less than 20 years old, and increased in older stubjects, being more than 70% in groups of over 30 years old.

3. *Incidences of HBeAg and anti-HBe in HBV carriers with LC and HCC*

The incidences of HBeAg and anti-HBe in the sera of patients with LC and HCC are shown in Table 2. HBeAg was found in 12 (27.3%) and anti-HBe in 27 (61.4%) of 44 patients with LC. Among 29 patients with HCC, 7 (24.1%) had only HBeAg and 18 (62.1%) had only anti-HBe; two (6.9%) had both HBeAg and anti-HBe and two (6.9%) had no detectable HBeAg or anti-HBe. There was no significant difference in the incidence of HBeAg or anti-HBe between individuals with and without LC and HCC ($\chi^2=0.64$, 0.11).

DISCUSSION

Epidemiologic studies have suggested a very close correlation between the geographical incidence of the HBV carrier state and HCC (Szmuness, 1978). In addition, a much incidence of HBsAg has been found in patients with HCC than in the general population (Coursaget et al., 1981; Chung et al., 1983). In a prospective study in Taiwan, the risk-factor ratio of HCC in HBV carriers was estimated to be as high as 200 to 300 times that in noncarriers (Beasley et al., 1981). In a 5-year follow-up study by Sakuma et al., 4 of 202 HBV carriers died of HCC and 3 died of other diseases (Sakuma et al., 1982). In our study on 336 HBV carriers for over 36 months (mean, 71 months), 2 died of HCC and none died of other diseases. In an investigation by Beasely et al., HCC and LC accounted for 57 (54.3%) of 105 deaths among HBV carriers (Beasley et al., 1981). In a study by Musca et al., 8 (42.1%) of 19 HBsAg-positive patients with LC developed HCC, whereas only 4 (7.8%) of 51 HBsAg-negative cases developed HCC (Musca et al., 1983). Thus although the precise incidences of HCC and LC among HBV carriers have not been defined, HBV carriers should be followed carefully for development of HCC and LC, particularly in areas of the world where the carrier rate of HBV is high.

Our study on the distributions of HBeAg and anti-HBe by age among 589 HBV carriers, showed that the incidence of HBeAg was about 60% in groups of less than 20 years old, less in the 20's group, and about 20% in groups of over 30 years old. In contrast, the incidence of anti-HBe was about 30% in groups of less than 20 years old, higher in the 20's group, and about 70% in groups of over 30 years old. These data suggest that seroconversion from HBeAg to anti-HBe occurs in more than 60% of HBV carriers before 30 years of age, and that the rate of seroconversion among individuals of more than 30 years old is relatively low. In our follow-up study on 139 HBeAg-positive individuals for more than 36 months, the rate of seroconversion was 3.6% per year without significant difference between males (4.1%) and females (2.7%). The age distribution of the time of seroconversion ranged between 6 and 79 years (mean, 42 years). Of 139 individuals, 58 were under 30 years old at the start of the follow-up study, and 81 were over 30 years old (mean, 36 years). Long-term studies on younger HBV carriers might indicate a higher rate of seroconversion from HBeAg to anti-HBe. In the follow-up study by Realdi et al. over a period of 2 to 7 years (mean, 3.6 years), 10 of 29 HBeAg-positive individuals showed seroconversion to anti-HBe (Realdi et al., 1980). In the longitudinal study of Hoofnagle et al. for 1 to 6 years (mean, 2.5 years), 13 of 25 HBeAg-positive individuals showed seroconversion to anti-HBe (Hoofnagle et al., 1981); these 25 individuals ranged from 21 to 60 years old (mean, 35 years). In the follow-up study of Liaw et al. of up to 60 months (mean, 24 months), 30 of 99 HBeAg-postitive individuals cleared HBeAg from their sera (Liaw et al., 1983);

these 30 individuals ranged from 20 to 52 years old (mean, 30 years). The difference in the observed rates of seroconversion might result from differences in ethnic groups, age, or sex, although our data did not show any difference between males and females.

It is known that the presence of HBeAg in the serum is correlated with the amount of circulating complete HBV particles and the level of HBV-specific DNA polymerase activity (Nordenfelt and Kjellen, 1975; Hindman et al., 1976; Nordenfelt and Andren-Sandberg, 1976; Takahashi et al., 1976; Werner et al., 1977; Ohori et al., 1979), and that HBeAg can be used as a marker of infectivity (Alter et al., 1976; Okada et al., 1976; Shikata et al., 1977). In addition, early studies suggested that in HBV carriers HBeAg was associated with progressive and severe liver disease, and that anti-HBe was associated with the healthy carrier state (Eleftheriou et al., 1975; El Sheikh et al., 1975; Trepo et al., 1976). However, HBeAg was found even in asymptomatic carriers with normal liver histology and anti-HBe was not uncommon in patients with chronic active hepatitis (Trepo et al., 1976; Smith et al., 1976; Dormeyer et al., 1981). Several studies have indicated high incidences of HBeAg and anti-HBe in patients with LC and HCC (Werner et al., 1976; Coursaget et al., 1978; Coursaget et al., 1981; Chung et al., 1983): Coursaget et al. observed that 25 (29.8%) of 84 HBsAg-positive patients with HCC had HBeAg and 50 (59.5%) had anti-HBe. Chung et al. found that 38% of 87 HBsAg-positive patients with HCC had HBeAg; since this rate was higher than in chronic HBsAg-positive control patients, Chung et al. postulated that patients with HCC were more likely to remain HBeAg-positive and that a prolonged replicative phase of chronic HBV infection might result in a predisposition for development of HCC. In our study, HBeAg was found in 12 (27.3%) and anti-HBe in 27 (61.4%) of 44 patients with LC. Among 29 patients with HCC, HBeAg was detected in 9 (31.0%) and anti-HBe in 20 (69.0%). There was no significant difference in the incidences of HBeAg and anti-HBe in HBV carriers with and without LC and HCC ($\chi^2=0.64,\ 0.11$). This suggests that the presence of anti-HBe in serum is not correlated with the healthy carrier state. However, HCC developed in 2 HBV carriers who showed seroconversion from HBeAg to anti-HBe. It is interesting that in these patients HCC was detected at the time of seroconversion from HBeAg to anti-HBe. This suggests that active replication of HBV sometimes becomes defective during the course of malignant transformation (Coursaget et al., 1978; Musca et al., 1983).

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