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CASE REPORT

Dynamics of hepatitis B virus serum markers in an acute hepatitis B patient in the incubation phase

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Abstract

Patients with acute hepatitis B (AHB) usually present after developing symptoms; therefore, the temporal kinetics of viral markers during the incubation period have not been documented clearly. We describe an AHB infection before the onset of hepatitis, throughout the course of the disease and without anti-viral therapy. The patient initially visited our hospital for immunization against HBV and was found to be positive for viral markers: 0.0 IU/mL of anti-HBs, 0.06 S/CO of anti-HBc and 2.93 IU/mL of HBsAg. During the 14 days after his first visit, HBsAg, HBV DNA, HBe antigen and HBV core-related antigen, but not anti-HBc or anti-HBs, levels increased. On day 22, he developed acute hepatitis. The period of logarithmic viral replication was estimated to be 7.0 days. HBV genomic sequencing and phylogenetic analysis indicated transmission from the patient's wife. Although sexual intercourse could not be ruled, another possible route of transmission was the unusual occurrence of kissing his wife when she had macroscopic bleeding after tooth brushing, 2 months before his positive HBsAg result; the day of the episode being consistent with the calculated HBV replication velocity. This study reveals the temporal kinetics of viral markers during the incubation period of AHB.

Keywords Acute hepatitis · HBV · HBsAg · Incubation phase

Introduction

Hepatitis B virus (HBV) infection is one of the most common persistent viral infections in humans. However, very few studies of the early phase of acute hepatitis B (AHB) in adults have been reported [1]. Because patients are usually diagnosed only after the development of symptoms, serum HBV markers in the incubation phase characterized by viremia, but with normal serum aminotransferase levels, have been documented rarely [2].

A figure demonstrating the dynamics of HBV serum markers in acute hepatitis B patients often is reproduced in textbooks; this shows that HBV DNA, followed closely by HBsAg and hepatitis B e antigen (HBeAg), are the first

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viral markers detected in serum, but the cited literature is quite old [3, 4]. Serological tests currently available for the various markers of HBV infection have improved sensitivity and specificity.

Here, we report the clinical course of a patient who was initially diagnosed during the incubation phase of AHB and followed up throughout the course of the disease, with detailed documentation of the viral markers and without anti-viral therapy.

Case report

This study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its subsequent amendments and with the informed consent of the patient and his wife to their inclusion in the study.

A healthy man in his 30 s, who was born and raised in Japan, visited our hospital for immunization against HBV. He had neither any medical history nor any medications in the past several years. He reported no alcohol consumption. On physical examination, he had no signs of chronic liver disease, jaundice, encephalopathy or hepatomegaly. There were no hepatitis B carriers in his family, except for his wife, who was positive for HBeAg, and whom he had married 2 years previously. As he was aware of the risk of sexual transmission of HBV, he had used condoms for prophylaxis whenever necessary. An unusual event as a possible risk of HBV transmission, other than through sexual intercourse, had occurred 2 months previously when he had kissed his wife while she had macroscopic bleeding after tooth brushing. As he and his wife hoped to have a baby, he consulted us for immunization against HBV.

At his initial visit to our hospital, we tested his serum for three markers to determine the indication for immunization against HBV: anti-hepatitis B surface antibody (anti-HBs, chemiluminescence immunoassay (CLIA)), anti-hepatitis B core antibody (anti-HBc, CLIA) and hepatitis B surface antigen (HBsAg, CLIA). He visited our hospital again 7 days later to be informed of the results: 0.0 IU/mL of anti-HBs, 0.06 S/CO of anti-HBc and 2.93 IU/mL of HBsAg. Based on the low but positive HBsAg result, probably before the induction of antibody after infection, we examined his serum from that day in more detail. Although no signs of hepatitis were apparent, with normal hepatic enzymes and total bilirubin, serum HBV DNA and HBeAg were detected as 5.1 Log IU/mL and 12.4 S/CO, respectively. Antibody against HBeAg (anti-HBe) and IgM anti-HBc, on the other hand, were found to be 0.0 INH% and 0.07 S/CO, respectively. The HBV genotype was C, according to an enzyme-linked immunosorbent assay (ELISA) [5]. A diagnosis of acute infection with HBV during the incubation period was confirmed. Physical examinations and blood tests were performed every week thereafter. Serum samples, including from the initial visit, were stored at − 20 °C with informed consent for the subsequent analysis of the HBV DNA sequence.

Clinico-virological features of the patient are illustrated in Fig. 1 and Table 1. During the 14 days after his initial visit,

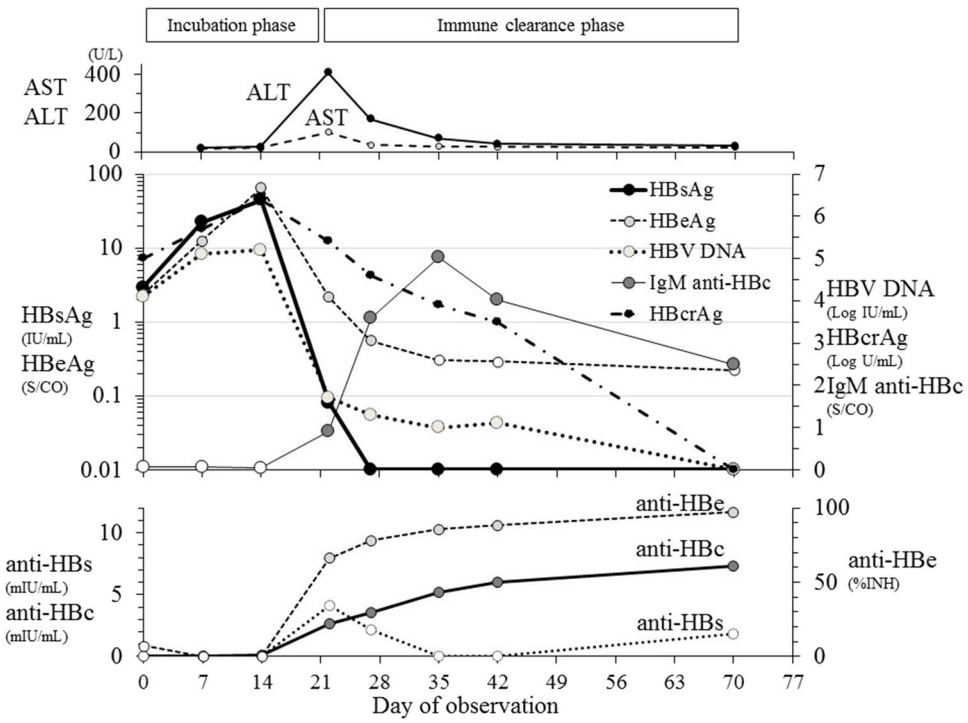

Fig. 1 The time course of HBV serum markers in the patient

Table 1 Time course of HBV serum marker values in the patient

Day after initial visit	0	7	14	22	27	35	42	70
AST (U/L)	Not tested	18	21	102	36	29	25	22
ALT (U/L)	Not tested	20	26	410	169	69	41	30
HBV DNA (Log IU/mL)	4.1	5.1	5.2	1.7	1.3	1.0	1.1	0.0
HBsAg (IU/mL)	2.93	22.19	44.85	0.08	0.01	0.01	0.01	0.01
HBeAg (S/CO)	2.4	12.4	64.8	2.2	0.6	0.3	0.3	0.2
HBcrAg (Log U/mL)	5.0	5.7	6.4	5.4	4.6	3.9	3.5	0.0
IgM anti-HBc (S/CO)	0.06	0.07	0.05	0.91	3.58	5.02	4.03	2.48
Anti-HBs (mIU/mL)	0.0	0.0	0.0	4.1	2.2	0.0	0.0	1.8
Anti-HBe (%INH)	7.2	0.0	0.0	66.4	78.2	85.7	88.5	97.4
Anti-HBc (mIU/mL)	0.06	0.07	0.10	2.63	3.57	5.21	6.04	7.30

the HBsAg, HBV DNA, HBeAg, HBV core-related antigen (HBcrAg), but not anti-HBc or anti-HBs, levels increased. On day 22, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and IgM anti-HBc were increased for the first time, while the HBsAg, HBV DNA, HBeAg and HBcrAg levels were in decline. The patient's infection likely was progressing from the incubation period to the immune-clearance phase around that day. The transaminase levels decreased thereafter and the HBsAg and HBeAg levels declined to below the detection limit on days 28 and 35, respectively. Both the HBV DNA and HBcrAg levels were positive until day 42 and then declined to below the detection limit on day 70. No blood tests were available from days 49 to 63. The peak IgM anti-HBc value was 5.02 S/CO on day 35. Anti-HBe and anti-HBc became positive from day 21 and increased gradually, but anti-HBs was not detected for 70 days. The patient was not prescribed anti-viral drugs because he did not develop any symptomatic episodes.

The patient had no discernible risk factors for HBV infection, other than his chronically infected spouse. She was in her 30 s and had been an outpatient in our hospital for 3 years, with a diagnosis of persistent HBV infection before the immune-clearance phase; her liver function tests and serological HBV markers were examined at each visit. Her blood test, obtained on the same day as the initial visit of the patient, revealed the following: total bilirubin (T.Bil), 0.3 mg/dL; AST, 13 U/L; ALT, 13 U/L; WFA(+)-Mac-2 binding protein, 0.3 COI; HBsAg, 7418 IU/mL; HBeAg, 1523 S/CO; anti-HBe, 0.0 INH%; HBV DNA, 8.3 Log IU/mL; HBV precore, wild type (G1896); basal core promotor, wild type (A1762/G1764); Genotype, C. Liver transaminases were persistently normal over the 3 years.

To confirm the source of infection, we carried out complete HBV genome sequencing for the patient and his wife, according to the method described previously [6]. The nucleotide sequence data have been deposited in the DDBJ/EMBL/GenBank databases under the accession numbers LC373511-LC373512. The HBV isolates from the patient and his spouse had a nucleotide sequence identity of 99.94% (3213 / 3215 bases), with only two base mismatches (nt 27, nt 3162) over the entire genome, and were classified into subgenotype C2. A phylogenetic tree of full-length HBV, obtained using the neighbor-joining method [7], revealed that the two isolates in this study were most closely related to each other (Fig. 2). Consequently, the wife of the patient was confirmed to be the source of infection.

We calculated the replication velocity in the incubation phase (0–7 days) of this case. The period of logarithmic viral replication in the patient was estimated to be 7.0 days, because the HBV DNA levels increased from 4.1 Log IU/mL to 5.1 Log IU/mL during the first 7 days.

Discussion

We evaluated the serum markers of an AHB patient in the incubation phase. In the incubation period of 14 days, during which the serum HBV DNA levels increased in the presence of completely normal ALT levels, the levels of HBsAg, HBeAg and HBcrAg were elevated without the induction of IgM anti-HBc, anti-HBe or anti-HBs. Conversely, in the immune-clearance phase, the levels of HBsAg, HBeAg and HBcrAg were declining and became undetectable in that order, with reciprocal induction of IgM anti-HBc and anti-HBe. AHB in the incubation period should be considered as a differential diagnosis in patients with positive HBsAg and normal serum aminotransferases.

The temporal kinetics of HBcrAg in the incubation and immune-clearance phases have been reported rarely. The HBcrAg level increased in parallel with HBsAg and HBV DNA in the incubation phase.

The HBV genome sequences and phylogenetic analysis indicated transmission to the patient from his wife. Although sexual intercourse is a well-documented, major route of horizontal transmission of HBV, it was not likely in this case; the patient had always used condoms for prophylaxis. From the interview to investigate an alternative transmission route, we found that the patient had an episode of kissing his wife

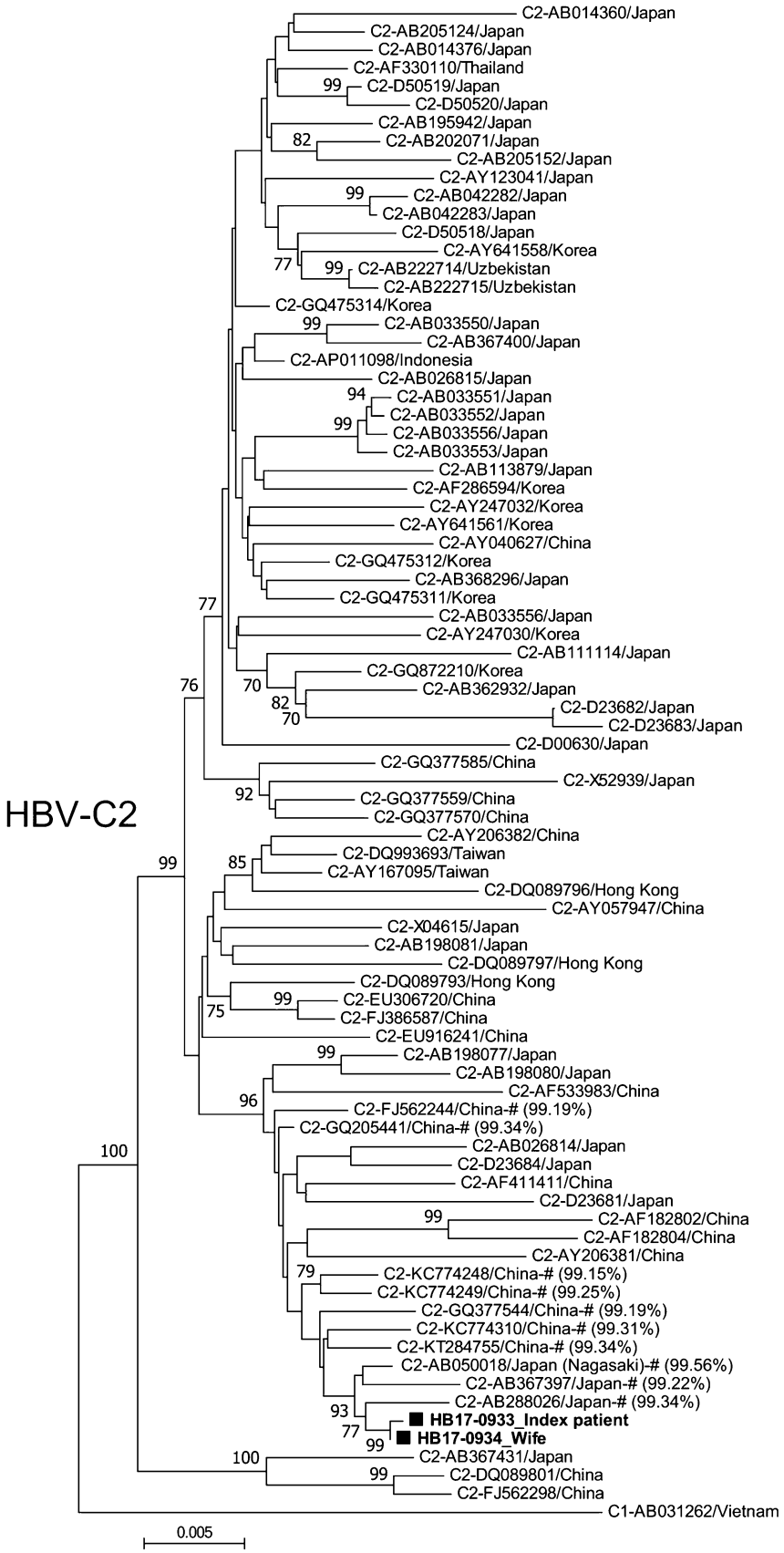

Fig. 2 Phylogenetic tree of full-length genotype C2 HBV strains, constructed using the neighbor-joining method, with a representative genotype C1 HBV strain (AB031262) as the outgroup. HBV isolates obtained from the patient and his chronically infected wife are shown in bold, with closed squares

when she had macroscopic bleeding after tooth brushing, 2 months before he had a positive HBsAg result.

HBV DNA is detectable in the saliva of a high proportion of chronic HBV carriers [8] and the infectivity of saliva from HBV carriers has been proven by experimental transmission, using gibbons [9, 10]. The oral administration of serum from HBV carriers, on the other hand, also has been documented to lead to infection, although the portal of entry is not clear, perhaps via an oral membrane or through the gastrointestinal tract [11]. Despite 3 years of normal married life, with sexual intercourse and close contact, anti-HBc was negative in the patient at the initial visit. Not only that the patient's wife's saliva contained HBV, but that it was contaminated with her blood after the accidental oral injury, likely led to transmission in this case.

The period of logarithmic viral replication in the incubation phase in this patient was estimated to be 7.0 days. Komiya et al. [12] reported that the logarithmic periods of HBV genotype C replication were 5.58, 5.96 and 8.31 days in three experimentally inoculated chimpanzees, 6.3 days (95% CI 5.41–7.54) in average. The replication period of 7.0 days in our patient was within 95% CI of the data from these chimpanzees. The HBV DNA level of our patient at the initial visit was 4.1 Log IU/mL. Two of the three chimpanzees who had similar HBV logarithmic periods of 5.96 and 8.31 days, to our patient with 7.0 days, had levels of 4.1 Log IU/mL for 49 days and 63 days after inoculation, respectively. The unusual event, when the patient had kissed his wife with bleeding gums, had occurred 2 months (about 56 days) earlier from the initial visit, that is within a very similar time-frame to the incubation periods calculated for the two chimpanzees. HBV, a blood-borne pathogen, may have a particular replication velocity in primates, including humans. Incubation period of this patient from the viral penetration (56 days before the initial visit) till the development of hepatitis (21 days later after the initial visit) was finally considered as 67 days.

In conclusion, this study documented the temporal kinetics of viral markers during the incubation period of an AHB patient. AHB in the incubation period should be considered as a differential diagnosis in patients with positive HBsAg and normal serum aminotransferases.

Compliance with ethical standards

Conflict of interest Shota Okamoto, Kazumi Yamasaki, Atsumasa Komori, Seigo Abiru, Shinya Nagaoka, Akira Saeki, Satoru Hashimoto, Shigemune Bekki, Hiroaki Okamoto and Hiroshi Yatsuhash1 declare that they have no conflict of interest.

Human rights All procedures followed have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Informed consent was obtained from all patients for being included in the study.

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