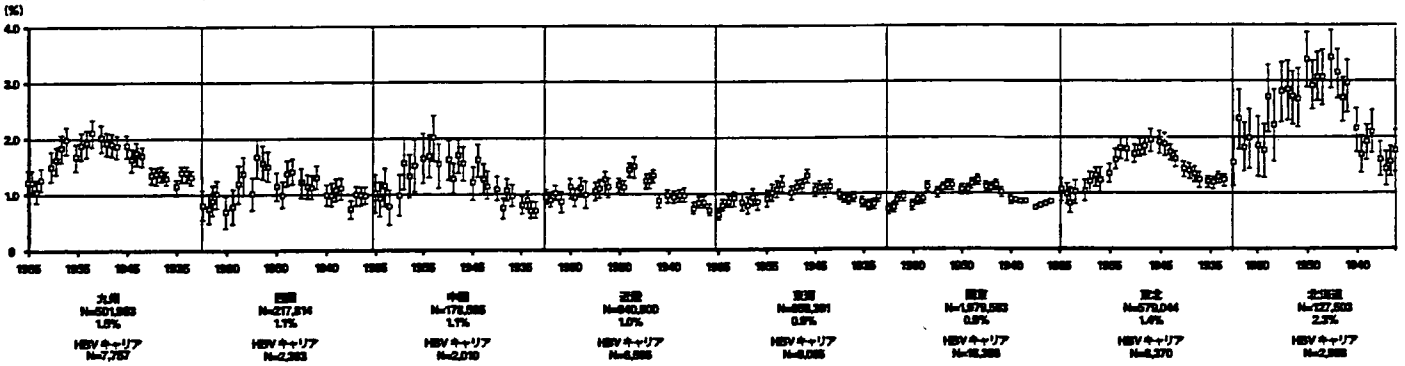
図5 地域別・出生年別にみたHBVキャリア率 ―節目検診受診者 2002年度～2005年度―



それぞれの集団におけるHBc抗体の有無を検出、測定した(表2)。その結果、前記の3つの集団から抽出したHBs抗体陽性例の中に占めるHBc抗体陽性率は、それぞれ81.9%、43.3%、11.0%と急激に減少していたことが明らかとなった(特に1992年以降に出生した群のみをみると、2.8～7.9%にまで激減)。すなわち、この調査からHBV母子感染防止事業の実施による母子感染(垂直感染)由来のHBVキャリアの減少に伴って、二次的にその前後の世代間でのHBVの水平感染が減少した結果、HBs抗体陽性者それ自体が減少し、しかもHBs抗体陽性例の大半はHBVの自然感染にはよらない、HBワクチンによる抗体獲得例に置き換わっていた。静岡県においても、HBV母子感染防止事業の実施に伴って、それ以降に出生した世代ではHBVキャリア率およびHBs抗体陽性率が激減している[6]。

■献血者集団におけるHBV感染の新規発生率

1994年6月から2004年4月までの間に、広島県赤十字血液センターにおいて献血した418,269人のうち、初回献血時のHBs抗原が陰性であり、かつ、調査期間内に2回以上献血した219,272人を対象としてHBV感染の新規発生率を調査した(図6)[7]。

HBV感染の「確診」(調査期間内にHBs抗原の陽転がとらえられ、かつ、HBc抗体価、HBs抗体価の推移、およびALT値の変動などから総合的に新規感染と判定可能であった)11例のみを新規感染例とみなした場合、HBV感染の新規発生率は10万人あたり年1.3人(95%CI:0.6～2.3人/10万人/年)となり、これに「疑診」(HBs抗原の陽転はとらえられたものの、その後の追跡ができておらず、HBc抗体価の推移も経時的に追うことができなかった)11例を含めた計22例を新規感染例とみなした場合でも、10万人あたり年2.5人(95%CI:1.6～3.9人/10万人/年)と、極めて低率に止まっている。なお、これらの22例の内訳をみると、男性は15例、女性は7例であり、感染時の年齢は22例中12例は20～29歳であった。

HBVの新規感染に関する調査を行う場合、検査の間隔が長い場合にはHBs抗原陽転の時期をとらえることができないことから、元来は上記の調査にHBs抗体陽転例、HBc抗体陽転例も加えて新規感染例であるか否かを確定するための検査を追加して行った上で、集計する必要があることはいうまでもないことである。しかし、調査対象集団が献血者であることから上述の調査方法に頼った。したがって、ここに述べた「HBVの新規感染」数は実態よりも低く推計されている可能性があることを付記しておく。

おわりに

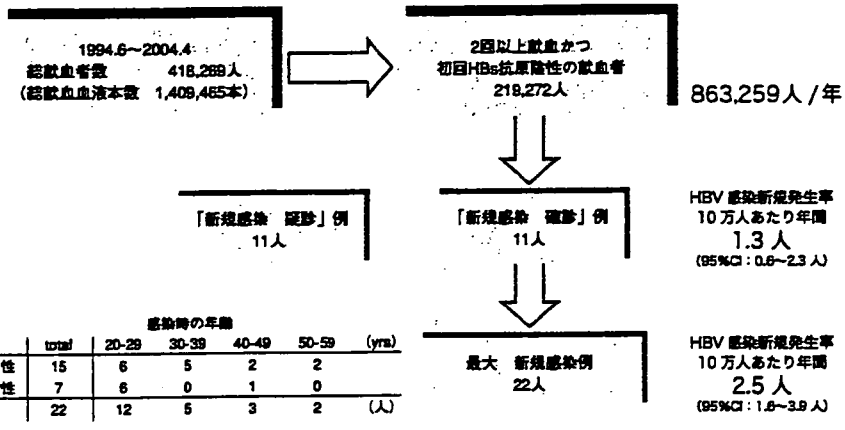
初回献血者集団、および肝炎ウイルス検診を受診した集団を対象として、年齢階級別、地域別にみたHBVキャリア率を解析した。その結果、現在のわが国では肝がんの好発年齢にあたる50歳から60歳(1945～1955年生まれの年齢層)におけるHBVキャリア率が他の年齢に比べて高い値を示すこと、地理的には東海、関東地区を除くすべての地区の40歳以上の年齢集団では単純平均が1%以上の比較的高い値を示すこと、特に北海道では2.3%と、とりわけ高い値を示す。

また、30歳から40歳(1965～1975年生まれ)の年齢層までは、いずれの地域においてもHBVキャリア率の大幅な減少はみられない。

一方、HBVの新規感染に関する調査、およびHBV母子感染防止事業実施前後に出生した児童を対象とした調査から、近年のわが国ではHBVの新たな感染は極めて低率にとどまっていること、とりわけ1986年からの母子感染防止事業の全面実施以降に出生した集団では、母子感染由来のHBVキャリアの減少に伴い、二次的に幼児期におけるHBV水平感染それ自体も激減していることが明らかとなった。

図6　献血者集団におけるHBV感染の新規発生率

広島県赤十字血液センター　1994.6～2004.4
総献血者数　418,269人
総献血血液本数　1,409,465本



感染時の年齢

	total	20-29	30-39	40-49	50-59	(yrs)
男性	15	6	5	2	2	
女性	7	6	0	1	0	
	22	12	5	3	2	(人)

表1　HBV母子感染防止事業実施前後に出生した児童におけるHBs抗原、HBs抗体陽性率

－岩手県予防医学協会－

出生年	児童数（人）	HBs抗原陽性数（%）	HBs抗体陽性数（%）
実施前（1978-1980）			
1978	2,668	26 (0.94)	52 (1.95)
1979	4,212	27 (0.64)	72 (1.71)
1980	3,559	25 (0.70)	35 (0.98)
小計	10,437	78 (0.75)	159 (1.52)
治験による予防（1981-1985）			
1981	2,541	12 (0.47)	30 (1.18)
1982	1,594	4 (0.25)	12 (0.75)
1983	3,847	6 (0.16)	17 (0.44)
1984	6,206	11 (0.18)	58 (0.93)
1985	6,624	13 (0.20)	48 (0.72)
小計	20,812	46 (0.22)	165 (0.79)
事業開始以降（1986-1990）			
1986	6,775	3 (0.04)	41 (0.61)
1987	6,505	4 (0.06)	62 (0.95)
1988	6,310	2 (0.03)	58 (0.92)
1989	6,436	2 (0.03)	64 (0.71)
1990	6,023	1 (0.02)	67 (1.11)
小計	32,049	12 (0.04)	292 (0.91)

調査年：1985-2000

表2　HBs抗体陽性の児童におけるHBc抗体陽性率　－岩手県予防医学協会－

出生年	HBs抗体陽性の児童数（人）	HBc抗体陽性数（%）
実施前（1978-1980）		
1978	49	40 (81.6)
1979	72	64 (88.9)
1980	34	23 (76.7)
小計	155	127 (81.9)
治験による予防（1981-1985）		
1981	30	23 (76.7)
1982	12	9 (75.0)
1983	14	6 (42.9)
1984	58	18 (31.0)
1985	43	12 (27.9)
小計	157	68 (43.3)
事業開始以降（1986-1990）		
1986	41	10 (24.4)
1987	61	11 (18.0)
1988	58	9 (15.5)
1989	46	6 (13.0)
1990	67	6 (9.0)
1991	62	7 (11.3)
1992	72	2 (2.8)
1993	63	5 (7.9)
1994	66	3 (4.5)
小計	536	59 (11.0)

調査年：1985-2002

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Case report

A male patient with severe acute hepatitis who was domestically infected with a genotype H hepatitis B virus in Iwate, Japan

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Although all eight genotypes of hepatitis B virus (HBV) strains are circulating in Japan, no cases of acute hepatitis with foreign HBV strains of genotype H have thus far been reported in Japan. Here, we report a 35-year-old Japanese patient with severe acute hepatitis who was domestically infected with genotype H HBV. On admission, he had a high HBV load of 1.0×10^9 copies/ml, elevated levels of total bilirubin (7.0 mg/dl) and alanine aminotransferase (3606 IU/l), and reduced prothrombin activity of 39.0%. The HB-JAIW05 isolate obtained in the present study was composed of 3215 nucleotides and had the highest similarity of 99.7% with the reported genotype H HBV isolate recovered from a Japanese blood donor. The HB-JAIW05 isolate had neither precore (A1896) nor core promoter (T1762/A1764) mutations. However, upon comparison with the consensus sequence of ten reported HBV isolates of the same genotype, the HB-JAIW05 isolate had 17 nucleotide substitutions including five missense mutations in the *P* gene, which may be related to vigorous replication of HBV in this case. He had no history of traveling abroad, but had had extramarital sexual contact with two Japanese women living in Iwate, Japan, 2 weeks and 2 months before the disease onset, respectively. Our results suggest that rare HBV genotypes such as H may be spreading in Japan via sexual contact. Further molecular epidemiological studies on HBV to clarify the exact changing profiles of de novo HBV infection in Japan in relation to genotype and genomic variability are warranted.

Key words: severe acute hepatitis, hepatitis B virus, genotype H

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Introduction

Hepatitis B virus (HBV) is one of the most important causes of acute and chronic liver diseases worldwide, including acute self-limited hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HBV possesses a circular, partially double-stranded DNA genome of approximately 3200 nucleotides (nt). It contains four major open reading frames encoding the envelope [preS1, preS2 and surface antigen (HBsAg)], precore/core antigen (HBeAg and HBcAg), polymerase (P), and X (HBx) proteins. Eight genotypes of HBV, A to H, have thus far been recognized,[1–4] and have different geographical distributions.[5–8] Genotype A is found predominantly in northwestern Europe, North America, and central Africa. Genotypes B and C are found in Southeast Asia, China, and Japan, whereas genotype D has a worldwide distribution but is predominant in the Mediterranean area, the Middle East, and India. Genotype E occurs frequently in Africa, whereas genotype F is found among American natives and in Polynesia and Central and South America. Genotype G HBV has been reported in France, Germany, the United States, and Mexico. The eighth genotype, named H, a newly described genotype, was considered to be confined to Latin America,[4] but it has been found not only in Nicaragua and Mexico but also in the United States and Japan.[4,9–13] Five of the genotypes, A, B, C, D, and F, have been further subdivided into subgenotypes, which are identified by Arabic numbers.[6,7]

In Japan, genotypes B and C are predominant, and genotypes A, D, and F are found in small percentages of HBV-viremic blood donors and patients with chronic HBV infection;[14,15] genotypes E, G, and H have rarely been found in patients infected with human immunodeficiency virus type 1 (HIV).[11] Since the successful

implementation of a nationwide program preventing mother-to-infant infection of HBV by administering hepatitis B immune globulins and HB vaccines to newborns[16] and nationwide nucleic acid amplification testing (NAT) of voluntarily donated blood for HBV, hepatitis C virus (HCV), and HIV by the Japanese Red Cross Blood Transfusion Services,[17,18] a major cause of acute HBV infection in Japan is sexual contact with HBV-viremic partners. A recent study indicated that genotype A HBV prevails among Japanese patients with acute hepatitis B, particularly in metropolitan areas of Japan.[19] However, no patients with acute hepatitis who were infected with foreign HBV strains of genotype H have thus far been reported in Japan. Here, we report a patient with severe acute hepatitis who was domestically infected with a genotype H HBV, most likely via sexual contact with Japanese women living in Iwate, a nonmetropolitan area located in the northern part of Honshu Island, Japan.

Case report

Methods

All routine hematological and biochemical examinations were performed using an autoanalyzer. The presence of hepatitis B surface antigen (HBsAg) and the corresponding antibody (anti-HBs), hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe), antibody to hepatitis B core (anti-HBc), IgM class anti-HBc and IgM class antibody to hepatitis A virus (IgM anti-HAV) was tested by chemiluminescence enzyme immunoassay using kits from Fuji Rebio (Tokyo, Japan). Third-generation antibody to HCV (anti-HCV) was measured by chemiluminescence immunoassay (Ortho Diagnostic Systems, Tokyo, Japan). IgG and IgM classes of antibodies to Epstein-Barr virus, cytomegalovirus, human parvovirus B19, human herpesvirus type 6, and varicella zoster virus were assayed by the fluorescent antibody method using the respective commercial kits.

The presence of HBV DNA was determined by polymerase chain reaction (PCR) with nested primers targeting the *S* gene of the HBV genome according to the method described previously.[20] The amplification product of the first-round PCR was 461 base pairs (bp), and that of the second-round PCR was 437 bp. Quantitation of HBV DNA was performed by real-time detection PCR using the LightCycler-FastStart DNA Master Hybridization Probes kit (Roche Diagnostics, Mannheim, Germany) as described previously.[21]

The presence of antibodies to hepatitis D virus (HDV) was determined by in-house enzyme-linked immunosorbent assay (ELISA), using purified recombinant S-HDAg protein that had been expressed in the pupae of silkworm, as described previously.[22] IgG, IgM, and IgA classes of antibodies to hepatitis E virus (HEV) (IgG anti-HEV, IgM anti-HEV and IgA anti-HEV, respectively) were assayed by in-house ELISA as described previously.[23]

The entire nucleotide sequence of the HBV genome was determined by methods essentially similar to those described previously.[24] Briefly, three overlapping regions of HBV DNA were amplified by nested PCR with TaKaRa *Ex Taq* (TaKaRa Bio, Shiga, Japan) and the appropriate primers derived from conserved areas of the genomes of the eight genotypes (A to H). The three overlapping regions (primer sequences at both ends excluded) that were amplified spanned nt 265–1795 (1531 bp), nt 1674–2380 (707 bp), and nt 2282–3215 and 1–458 (1392 bp). The amplification products were sequenced directly on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence analysis was performed using Genetyx-Mac version 12.2.5 (Genetyx, Tokyo, Japan) and ODEN version 1.1.1 from the DNA Data Bank of Japan (DDBJ: National Institute of Genetics, Mishima, Japan).[25] Phylogenetic trees were constructed by the neighbor-joining method.[26] Bootstrap values were determined on 1000 resamplings of the data sets.[27]

Profile and clinical course of the present case

The patient was a 35-year-old Japanese man. He noticed dark urine, general fatigue, and anorexia in August 2005. On the following day, he consulted a physician in private practice, and abnormalities on liver function tests were found. Two days later, he was hospitalized at a local hospital. However, his symptoms worsened, and his prothrombin activity was markedly low. He was transferred to the Iwate Medical University Hospital 2 days later. He had no history of blood transfusion, travel abroad, excessive intake of alcohol, or drug abuse. However, he had had extramarital sexual contact with two Japanese women living in Iwate Prefecture, Japan, 2 weeks and 2 months, respectively, before the onset of the disease.

On admission, he had severe jaundice in the bulbar conjunctiva and skin, but he was alert and there were no abnormal vital signs. Physical examination revealed no lymphadenopathy, hepatomegaly, splenomegaly, ascites, or edema. Laboratory findings on admission showed markedly elevated serum total bilirubin and liver enzyme levels, and low serum albumin and total cholesterol levels. Prothrombin activity was remarkably low, and the human hepatocyte growth factor level was moderately elevated (Table 1). Screening tests for hepatitis virus markers revealed positivity for HBsAg and

Table 1. Laboratory findings on admission

Parameter	Value	Parameter	Value
Hematology		Viral markers	
White blood cells	8230/μl	IgM anti-HAV (C.O.I.)	0.2 (–)
Red blood cells	487×10^4/μl	IgM anti-HBc (C.O.I.)	31.2 (+)
Hemoglobin	15.7 g/dl	HBsAg (C.O.I.)	>2000 (+)
Platelets	14.8×10^4/μl	Anti-HBs (C.O.I.)	0.5 (–)
Blood chemistry		HBeAg (C.O.I.)	319.2 (+)
Total protein	6.5 g/dl	Anti-HBe	71.6% (+)
Albumin	3.6 g/dl	Anti-HBc	100% (+)
Total bilirubin	7.0 mg/dl	Anti-HBc (1:200 dilution)	97.5% (+)
Direct bilirubin	5.4 mg/dl	HBV DNA (copies/ml)	1.0×10^9 (+)
AST	1679 IU/l	Anti-HCV (C.O.I.)	0.2 (–)
ALT	3606 IU/l	IgG anti-HDV (OD value)	0.014 (–)
LDH	643 IU/l	IgG anti-HEV (OD value)	0.014 (–)
ALP	527 IU/l	IgM anti-HEV (OD value)	0.203 (–)
γ-GTP	212 IU/l	IgA anti-HEV (OD value)	0.049 (–)
Total cholesterol	65 mg/dl	HEV RNA	(–)
IgG	1451 mg/dl	IgM antibody to EBV VCA	<1:10 (–)
IgA	176 mg/dl	IgG antibody to EBV VCA	1:80 (+)
IgM	330 mg/dl	Antibody to EBV EBNA	<1:10 (–)
NH_3	38 μg/dl	IgM antibody to CMV	<1:10 (–)
BUN	4.9 mg/dl	IgG antibody to CMV	1:10 (–)
Creatinine	0.6 mg/dl	IgM antibody to B19	(–)
Na	37 mEq/l	IgG antibody to B19	(+)
K	4.0 mEq/l	IgM antibody to HSV	<1:10 (–)
Cl	106 mEq/l	IgG antibody to HSV	1:80 (+)
Coagulation tests and others		IgM antibody to HHV-6	<1:10 (–)
Prothrombin time	18.8 s (39.0%)	IgG antibody to HHV-6	1:40 (+)
Fibrinogen	210.6 mg/dl	IgM antibody to VZV	(–)
hHGF	0.75 ng/ml	IgG antibody to VZV	(+)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; BUN, Blood urea nitrogen; hHGF, human hepatocyte growth factor; C.O.I., cutoff index; HAV, hepatitis A virus; anti-HBc, antibody to hepatitis B core; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; EBV VCA, Epstein-Barr virus viral capsid antigen; EBNA, Epstein-Barr nuclear antigen; CMV, cytomegalovirus; B19, human parvovirus B19; HSV, herpes simplex virus; HHV-6, human herpesvirus type 6; VZV, varicella zoster virus; OD, optical density

IgM anti-HBc, but negativity for anti-HBs antibody. HBeAg and anti-HBe antibody were both positive. The HBV DNA titer in the circulation was markedly high at 1.0×10^9 copies/ml. IgM antibodies against hepatitis A, C, D, and E viruses and other viruses, including Epstein-Barr virus, cytomegalovirus, human parvovirus B19, herpes simplex virus, human herpes virus type 6, and varicella zoster virus, which are known to be related to hepatic injury, were all negative (Table 1). Furthermore, anti-nuclear antibody and anti-mitochondria antibody were negative. Abdominal computed tomography and ultrasonography showed no signs of hepatic failure such as liver atrophy, irregular density of the liver, or ascites, but did reveal slight splenomegaly and the collapse and thickening of the wall of the gallbladder, which are found in patients with acute hepatitis. Based on these results, he was diagnosed as having severe acute hepatitis B due to de novo HBV infection.

Figure 1 illustrates the clinical course of the patient after admission. Although he did not receive artificial liver support or any antiviral drug, his consciousness level and general condition did not deteriorate and his transaminase levels and prothrombin activity gradually improved. Laparoscopy performed on day 22 of admission showed no macroscopic abnormalities of the liver. Histological examination of the biopsied liver specimens showed slight infiltration of mononuclear cells in the portal area unaccompanied by fibrosis. He was discharged on day 33. During the course of his illness, the serum HBV DNA level gradually decreased and became undetectable (<30 copies/ml) on day 159. HBsAg became negative on day 86.

Analysis of the full-length genomic sequence of HBV

The full-length genomic sequence of an HBV isolate (HB-JAIW05) obtained from the present case was determined and deposited in the DDBJ/GenBank/EMBL databases (accession no. AB266536). The HB-JAIW05 isolate had a total genomic length of 3215 nt, which is

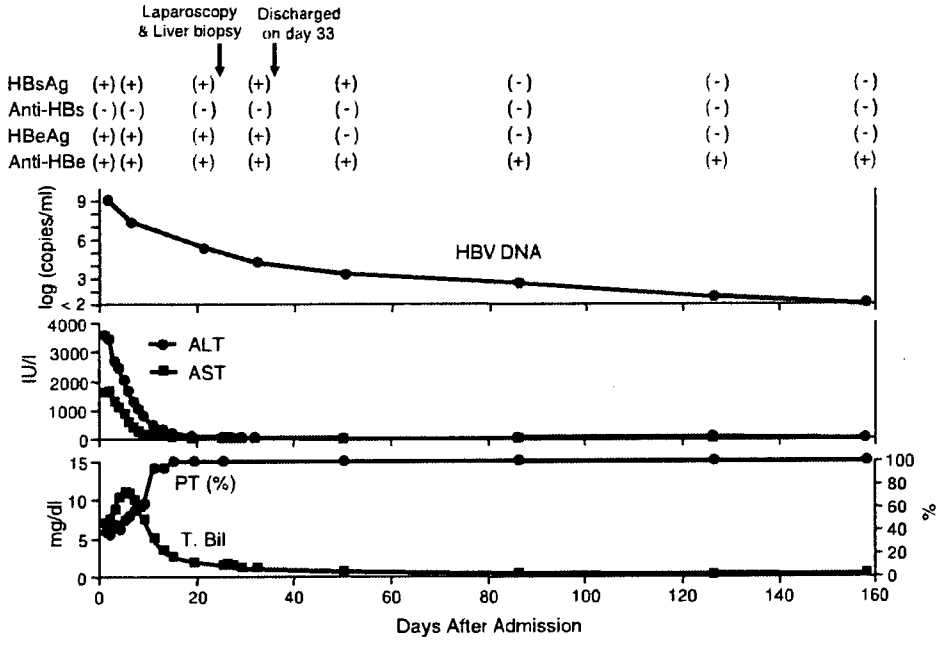

Fig. 1. Laboratory parameters and hepatitis B virus markers in serum samples that were periodically obtained from the patient with severe acute hepatitis B. The patient was admitted to our hospital on day 0 and discharged on day 33. *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *PT*, prothrombin activity; *T. Bil*, total bilirubin; HBsAg, hepatitis B surface antigen; Anti-HBs, antibody to hepatitis B surface antigen; HBeAg, hepatitis B e antigen; Anti-HBe, antibody to hepatitis B e antigen

identical to that of reported HBV isolates of genotypes B, C, F, and H. When the entire nucleotide sequence was compared with the reported genomes of all eight genotypes for which the full-length sequence is known, the HB-JAIW05 isolate was most closely related to the genotype H isolates with identities of 97.4%–99.7%, was close to the genotype F isolates with identities of 91.0%–92.8%, but was only 84.5%–86.8% identical to the HBV isolates of the remaining six genotypes (A, B, C, D, E, and G). Among the ten genotype H strains isolated in the United States, Nicaragua, and Japan whose full-length sequence has been determined, the HB-JAIW05 isolate had the highest similarity of 99.7% with the HB-JBDH1 isolate recovered from a Japanese blood donor whose viremia was identified by nucleic acid amplification testing and who presumably donated blood during the serological window period at an early stage of de novo infection.[12]

A phylogenetic tree was constructed based on the entire genomic sequence of 53 HBV isolates (including the HB-JAIW05 isolate obtained in the present study), which confirmed that the HB-JAIW05 isolate segregated into genotype H (Fig. 2). Close relatedness of HB-JAIW05 with the reported HBV strains of genotype H and clear branching of the eight genotypes were observed.

The HB-JAIW05 isolate had neither precore (preC, nt 1896) nor core promoter (nt 1762, 1764) mutations (A1896, T1762/A1764 mutation, respectively). Upon comparison with the consensus sequence of ten reported HBV isolates of the same genotype (for accession nos., see Fig. 2), the HB-JAIW05 isolate had 17 nucleotide substitutions that resulted in five amino acid substitutions in the *P* gene product, two amino acid substitutions in the *X* gene product, and one each in the preS2 region and *S* gene products (Table 2).

Discussion

In the present study, we reported a 35-year-old Japanese man with severe acute hepatitis who was infected with a genotype H HBV and had never been abroad. In Japan, a recent nationwide survey revealed the frequencies of various HBV genotypes among 301 patients with acute hepatitis B: genotype C was most prevalent (67%), followed by genotype B (15%), genotype A (14%), genotype G (2%), and genotype D (2%).[28] In addition to this large-scale survey, many reports on the distribution of HBV genotypes among patients with acute hepatitis in Japan have recently been published;[19,29–33] however, there have been no patients with acute hepatitis who were infected with a genotype H HBV, suggesting that this is the first report of an acute hepatitis patient in Japan who contracted infection of a genotype H HBV domestically.

Eight genotypes of HBV are currently recognized.[1–4] Each HBV genotype shows a distinct geographical distribution between and even within regions, and such data are an invaluable tool in tracing the molecular evolution and patterns and modes of spread of HBV.[5–8] Among the eight genotypes, genotype H has most recently been identified from two Nicaraguans and one American living in Los Angeles,[4] and has also been

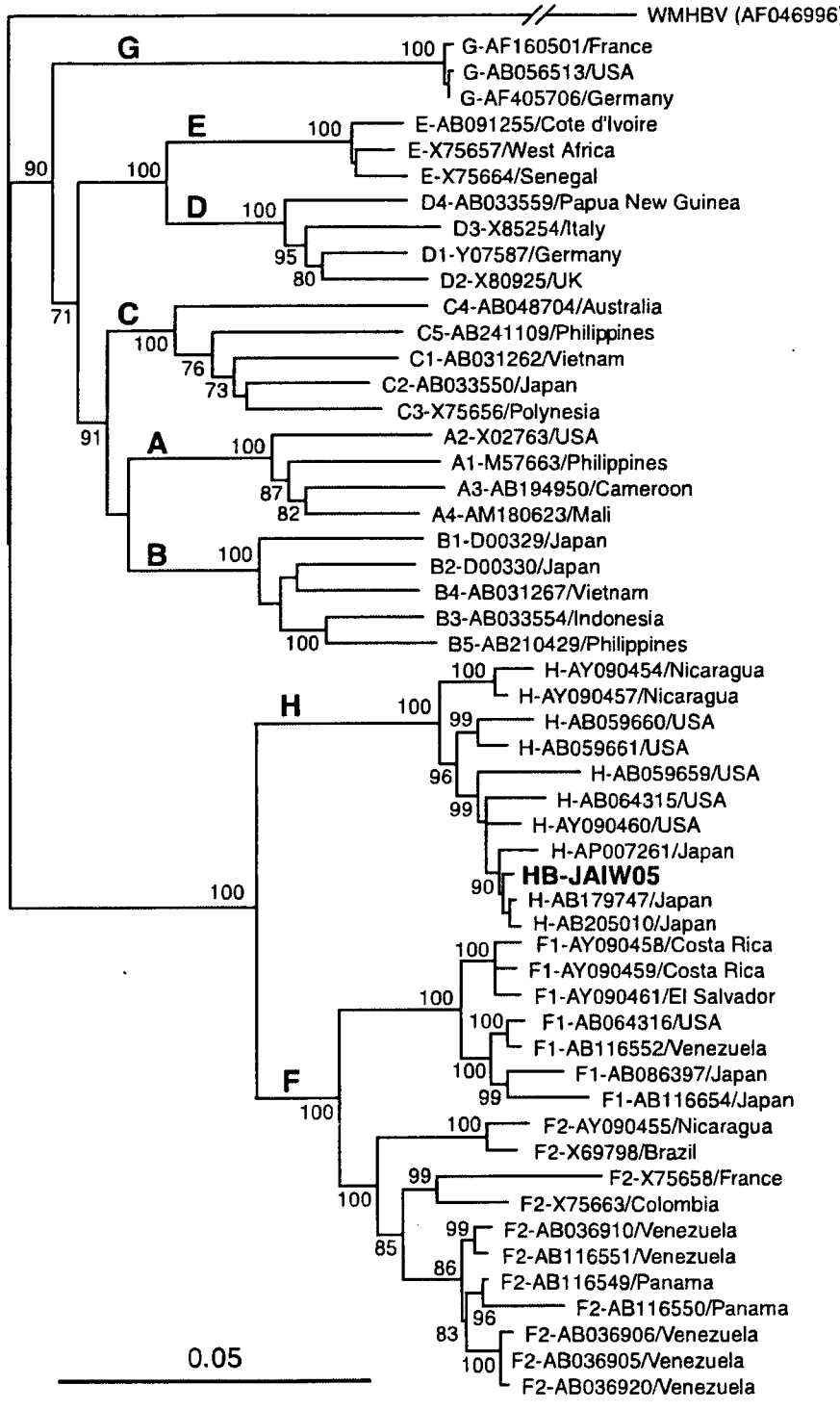

Fig. 2. Phylogenetic tree constructed by the neighbor-joining method based on the entire nucleotide sequence of 53 hepatitis B virus (HBV) isolates, using a woolly monkey HBV isolate (WMHBV: AF046996) as an out-group. In addition to the HB-JAIW05 isolate obtained from the patient with severe acute hepatitis B in the present study, indicated in bold type for visual clarity, 24 representative HBV isolates of genotypes *A* to *E* and *G* and all 18 genotype *F* and 10 genotype *H* isolates whose entire sequence is known were included for comparison. The previously reported isolates are indicated with the genotype or subgenotype and accession no., followed by the name of the country where it was isolated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings

encountered in Mexico and San Francisco.[9,10] However, the nature of HBV genotype H throughout the world remains obscure. In Japan, genotype H is very rarely found, and only three genotype H isolates have thus far been reported. Shibayama et al.[11] reported that a genotype H strain (HB-JI260; accession no. AP007261) was isolated from a 46-year-old Japanese patient coinfected with HIV who had a history of traveling to South America and had had a sexual relationship there.[11] Nakajima et al.[13] isolated a genotype H strain (HBV-IM806-2; AB205010) from a 61-year-old Japanese patient with chronic hepatitis, who while visiting Bangkok, Thailand, 30 years earlier had had a sexual relationship with a woman there, and 3 months after returning to Japan acquired acute hepatitis, which developed into chronic hepatitis B. In addition, the

Table 2. Sequence comparison between the HBV clone from this case and the consensus sequence from ten reported HBV clones of the same genotype (genotype H)

Nt position	Nt change	Genomic region	Amino acid change
46	T to G	preS2, P	__, Ser319 to Ala319 (P)
147	C to T	preS2, P	Ala53 to Val53 (preS2), __
242	C to A	S, P	Gln30 to Lys30 (S), Thr384 to Lys384 (P)
747	T to C	S, P	__, __
851	T to C	P	__
868	T to C	P	__
870	A to T	P	__
1164	G to A	P	__
1287	A to C	P	__
1306	C to A	P	__
1386	C to T	P, X	__, __
1504	C to T	P, X	Pro805 to Ser805 (P), Ala44 to Val44 (X)
1632	C to T	X	Arg87 to Trp87 (X)
2035	C to A	C	__
2101	C to A	C	__
2410	A to T	C, P	__, His35 to Leu35 (P)
2505	G to A	P	Val67 to Ile67 (P)

Nt, nucleotide

Japanese Red Cross NAT Screening Research Group reported that HBV genotype H has been found in only one (0.3%) of 328 HBV DNA-positive blood donors in Japan, and that the genotype H isolate (HB-JBDH1; AB179747) was recovered from a 52-year-old Japanese man who donated blood in October 2002.[12] These results and our present result indicate that individuals infected with a genotype H HBV are rare, but may serve as possible infectious sources in Japan.

The number of cases of acute HBV infection in Japan is estimated to be over 10000 cases per year. More than 50% of patients with acute hepatitis B had extramarital sexual contact within a period of time corresponding to the incubation period before the development of acute hepatitis B,[34] indicating that a major cause of acute HBV infection in Japan is sexual intercourse with an HBV-viremic partner. The precise reason why genotype A HBV has become prevalent among Japanese patients with acute hepatitis B remains unknown. However, the decreasing rates of mother-to-baby transmission and transfusion-associated transmission of genotypes B and C along with the increasing risk of sexual transmission may be involved in the changing pattern of the distribution of HBV genotypes in Japan, and rare HBV genotypes that are not found in chronic HBV carriers would become prevalent among individuals with high sexual activity with diverse partners, some of whom may be infected with various blood-borne viruses, including HIV and HBV, and even among individuals with sexual relationships with commercial sex workers, who are at high risk for contracting infection of blood-borne viruses. The present patient had extramarital sexual contact with two Japanese women living in Iwate, who were not commercial sex workers, 2 weeks and 2 months before the disease onset, respectively, although it remains unknown whether they were infected with HBV. The present case occurring in a nonmetropolitan area suggests that rare HBV genotypes such as genotype H may be widely distributed in Japan. Extensive molecular epidemiological surveys on the distribution of HBV genotype are needed to clarify the exact changing profile of de novo HBV infection in Japan and to develop programs to prevent acute HBV infection.

Severe acute hepatitis is defined as acute hepatitis having reduced prothrombin activity of <40% but without overt hepatic encephalopathy (coma grade > II). Our case had a prothrombin activity level of 39% on admission, but, fortunately, he did not develop hepatic encephalopathy. A nationwide survey that evaluated the outcome of severe acute hepatitis indicated that 31% of 164 patients with severe acute hepatitis developed overt encephalopathy and were diagnosed as having fulminant hepatic failure.[34] Therefore, prothrombin activity is one of the most important markers for predicting the development of hepatic encephalopathy in patients with acute hepatitis. Besides prolonged prothrombin time, old age and an elevated total bilirubin level were estimated to be potential risk factors for developing encephalopathy among patients with severe acute hepatitis.[34] However, our patient was young (35 years old) and his total bilirubin level was only moderately elevated (7.0 mg/dl). These findings may be consistent with his good clinical course. Recent studies have shown that the genotype of HBV is closely associated with the pathogenesis and clinical outcome of HBV-related liver diseases.[35–37] Viral factors may also play a

role in the pathogenesis of severe acute hepatitis or fulminant hepatitis. HBV variants with mutations in the precore region (A1896) and/or the core promoter (T1762 and A1764) have been implicated in fulminant hepatitis.[38-42] It is possible that the frequency of genomic mutations differs according to genotype. However, the particular genomic mutations in genotype H HBV that are associated with the severe or fulminant form of acute hepatitis or exacerbation of chronic liver disease are not known. Although the HB-JAIW05 isolate was recovered from a patient with severe acute hepatitis B in the present study, neither precore mutation (A1896) nor double mutations in the core promoter region (T1762/A1764) were present. When compared with the consensus sequence of all ten reported genotype H HBV strains whose entire sequence is known, including two Nicaraguan isolates, five Californian isolates, and three Japanese isolates, 17 nucleotide substitutions unique to the HB-JAIW05 isolate were recognized. The 17 nucleotide changes included five missense mutations (amino acids 35, 67, 319, 384, and 805) in the *P* gene, which may be associated with vigorous replication of HBV as observed at the time of admission of the present patient (1.0×10^9 copies/ml). Although further studies on a greater number of patients with acute hepatitis who are infected with genotype H HBV are required, these 17 nucleotide substitutions in HBV DNA could be candidates for mutations associated with the severe or fulminant form of acute hepatitis.

In conclusion, we identified and determined the full-length sequence of a genotype H HBV (HB-JAIW05) that had been recovered from a 35-year-old Japanese patient with severe acute hepatitis, who was presumed to have contracted the disease via sexual contact with Japanese women living in Iwate, a nonmetropolitan area in the northern part of Honshu Island. Our results suggest that rare HBV genotypes such as H may be spreading in Japan via sexual contact. Further molecular epidemiological studies on HBV infection are warranted to clarify the changing profiles of de novo HBV infection in Japan in relation to genotype and genomic variability.

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Initial load of hepatitis B virus (HBV), its changing profile, and precore/core promoter mutations correlate with the severity and outcome of acute HBV infection

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Background. The pathogenesis of the fulminant or severe form of acute hepatitis B virus (HBV) infection remains unclear, although both host- and virus-specific factors are considered to have a great impact on the clinical course. We aimed to define possible viral factors implicated in the severe form of acute HBV infection. ***Methods.*** We investigated viral factors in 42 patients with acute HBV infection: 11 had fulminant hepatitis (FH); 9 had a severe form of acute hepatitis (SAH), defined as having a prothrombin activity of less than 40% without encephalopathy; and 22 had acute self-limited hepatitis (AH). ***Results.*** Although there was no significant difference in serum HBV DNA levels on admission among the three groups, the level decreased more rapidly in patients with SAH or FH than in those with AH. In patients with SAH or FH, the HBV load on admission was higher in patients who died than in those who recovered (7.0 ± 1.6 vs 5.6 ± 1.0 log copies/ml; $P = 0.0293$). In univariate analysis, seronegativity for hepatitis B envelope antigen (HBeAg) and mutations in both the precore (G1896A and/or G1899A) and core promoter (T1753A/C and/or T1754C/G and/or A1762T/G1764A) were associated with FH (odds ratio [OR], 5.60; $P = 0.0269$ and OR, 52.0; $P = 0.0006$; respectively). In multivariate logistic regression analysis, only the presence of precore/core promoter mutations was associated with FH (OR, 42.8; $P = 0.0020$). ***Conclusions.*** The rapid decrease in viral load in the early phase of acute HBV infection was associated with the severity of the disease. A high viral load on admission and the presence of both precore and core promoter mutations in patients with severe coagulopathy closely correlated with mortality.

Key words: fulminant hepatitis, serum HBV DNA level, precore/core promoter mutation

Introduction

Liver injuries caused by hepatitis B virus (HBV) broadly range from acute self-limited hepatitis (AH) to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Fulminant hepatitis (FH) B is rare; it occurs in approximately 1% of patients with acute hepatitis B[1] and has a mortality rate of up to 80%[2] without liver transplantation. The pathogenesis of FH in acute HBV infection remains unclear, although both host- and virus-specific factors are considered to have marked effects on the clinical course. FH in adults has been reported to be associated with mutations in the basic core promoter (A to T mutation at nucleotide [nt] 1762 [A1762T] and G1764A)[3] and the precore region (G1896A).[4,5] It was suggested that these mutations of HBV may have affected viral replication, altered HBV-protein expression, and induced more severe liver damage when HBV was experimentally inoculated into chimpanzees.[6,7] Further analyses suggested that genotype B isolates had more precore mutations than genotype C isolates[8–10] and such mutations were detected more frequently in patients with FH than in those with acute self-limited hepatitis B.[11]

In a prospective study of chronic HBV infection in the absence of interferon or nucleotide analogue therapy, the HBV DNA level in circulation was found to be associated with the progression of chronic hepatitis to cirrhosis, irrespective of the serum alanine aminotransferase (ALT) level and hepatitis B envelope antigen (HBeAg) status.[12] However, it has not been fully investigated whether the serum HBV DNA level in acute HBV infection is associated with the severity or outcome of the disease. There have been limited data on viral replication and viral load during FHB. To date, it

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has been reported that FH patients with detectable HBV DNA had a lower mortality rate than those without, and it has been considered that an enhanced immune response decreases the viral replication level,[1,3,14] suggesting that antiviral agents may not be useful for treating FH. In previous studies, HBV DNA assays were performed only after the onset of hepatic encephalopathy, not before it. Moreover, it has not been clarified yet how viral load and its changing pattern in the early phase are associated with the clinical outcome and whether the presence of precore or core promoter mutations correlates with viral load in patients with acute HBV infection.

In this study, we retrospectively quantified serum HBV DNA in 42 patients with acute HBV infection, the severity of which ranged from acute self-limited hepatitis to FH observed from the early phase of the clinical course, to assess whether viral load was an important factor for their prognosis, in relation to precore or core promoter mutations.

Patients and methods

Patients with acute HBV infection

Forty-two consecutive patients with acute HBV infection, who were admitted to Iwate Medical University Hospital from June 1990 to March 2005, were enrolled in this study. Patients who had been treated with antiviral drugs and those who had received blood transfusion (including plasma exchange) in a previous hospital after the disease onset were excluded from this study. Twenty-two patients were men, and the mean age ± standard deviation (SD) was 36 ± 17 years (range, 18–75 years). Aminotransferase activity peaked within 4 days after admission. Twenty-two patients had AH with a prothrombin activity equal to or greater than 40% of the control value during the clinical course; 9 patients had the severe form of acute hepatitis (SAH) with a reduced prothrombin activity of less than 40% of the control value, without hepatic encephalopathy during the clinical course; and 11 patients had FH with reduced prothrombin activity that was less than 40% of the control value. Signs of encephalopathy were present on admission in 9 patients and appeared within a week following admission in 2. Of these 11 patients, 7 patients became comatose within 7 days after the clinical onset of hepatitis. The appearance of early symptoms, such as fever, anorexia, malaise, nausea, vomiting, jaundice, and right hypochondrial discomfort, was defined as the clinical onset. Seven patients in deep coma and who did not undergo liver transplantation died of hepatic failure. The other 35 patients recovered spontaneously with a marked decrease in ALT activity and normal prothrombin activity.

Acute HBV infection was determined by either the appearance of hepatitis B surface antigen (HBsAg) or the detection of the high-titer immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc IgM). All 42 patients were negative for antibody against hepatitis D virus. Although one female patient with FH was positive for antibody to hepatitis C virus (HCV), she had no HCV RNA detectable by reverse transcription-polymerase chain reaction (RT-PCR) assay. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Iwate Medical University, and informed consent was obtained from each patient or a family member of the patient. Serum samples, periodically collected from 31 patients after we had obtained their informed consent, were stored at –70°C until assay.

Serological markers of HBV infection and quantitation of HBV DNA

The presence of HBsAg was determined by radioimmunoassay (Authria-II-125; Abbott Japan, Tokyo, Japan) or enzyme-linked immunosorbent assay (ELISA; AxSYM; Abbott Japan). The presence of HBeAg and the corresponding antibody (anti-HBe) was determined by ELISA (AxSYM; Abbott Japan) or by chemiluminescence, using commercial assay kits (ARCHITECT; Abbott Japan). IgM class anti-HBc was detected by radioimmunoassay (Authria-II-125) or by chemiluminescence, using commercial assay kits (ARCHITECT). Serum HBV DNA was quantified by real-time detection PCR assay based on Taq Man chemistry (Operon Biotechnologies, Tokyo, Japan), as described previously.[15] All HBV DNA determinations were analyzed after $\log_{10}$ transformation. The linear range of this assay was 2.3–9.0 log (copies/ml). Samples with values exceeding 9.0 log (copies/ml) were considered to be above the linear range of the assay and were retested after a tenfold dilution, using normal human serum.

Genotypes and subgenotypes of HBV

The seven major HBV genotypes (A to G) were determined by a genotype-specific probe assay, using commercial kits (SMITEST HBV Genotype Detection kit; Genome Science Laboratories, Fukushima, Japan). This assay depends on the PCR products of the preS1 region of the HBV genome (nt 2902–3091) detected by hybridization with genotype-specific probes immobilized on a microwell plate.[16] For untypeable samples, the HBV genotype was determined by phylogenetic analysis of the S gene sequence, as described previously.[17]

The subgenotypes of Bj (Japanese type) without recombination with genotype C over the precore region and the core gene and Ba (Asian type) with the recombination were determined by the restriction fragment-

length polymorphism method based on specific nucleotide substitutions, as previously reported.[18]

Determination of HBV sequences

The sequences of HBV genomes were determined by the direct sequencing method after PCR amplification. Several primers were prepared for amplifying the basic core promoter (nt 1742–1849) and precore regions (nt 1814–1900). The primers for the first-round PCR were 5′-GTC TGT GCC TTC TCA TCT GCC-3′ (sense, nt 1553–1573) and 5′-AGA ATA GCT TGC CTG AGT GC-3′ (antisense, nt 2060–2079), and the primers for the second-round PCR were 5′-ACG TCG CAT GGA GAC CAC CG-3′ (sense, nt 1603–1622) and 5′-GAA AGA AGT CAG AAG GCA AA-3′ (antisense, nt 1954–1973). In brief, 5 µl of DNA extracted from serum was added to 4 µl of dNTP, 0.25 µl of TaKaRa Ex Taq (TaKaRa Bio, Shiga, Japan), 5 µl of 10 × Taq polymerase buffer, and 20 pmol of outer sense and antisense primers. The amplification profile was 2 min at 95°C, followed by 30 cycles at 94°C for 15 s (denaturation), 60°C for 45 s (annealing), and 72°C for 45 s (extension). For the second-round PCR, 1 µl of the first PCR product was added to the same reaction buffer with the inner sense and antisense primers but without the outer sense and antisense primers. The second PCR products (10 µl) were analyzed by 3% agarose gel electrophoresis using a molecular marker, and then stained with ethidium bromide. Direct sequencing was performed using an ABI PRISM 3100-Avant Genetic analyzer (Applied Biosystems Japan, Tokyo, Japan). Sequencing analysis was carried out using GENETYX-WIN version 4 (Genetyx, Tokyo, Japan).

Statistical analysis

Biological, clinical, and virological parameters determined within 24 h of admission were analyzed. Values for results were expressed as means ± SD. Differences in clinical characteristics among the three patient groups were analyzed by analysis of variance and the χ^2 test. Differences between two groups were analyzed by the χ^2 test, Fisher's exact test, or Student's *t*-test. A *P* value of less than 0.05 was considered to be statistically significant. Univariate and multivariate logistic regression analyses were performed using STATVIEW 5.0 for Macintosh (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics

There were no significant differences among the three groups (AH, SAH, and FH) in age, sex, duration from onset to admission, history of alcohol intake, or treatment with antiviral and/or immunosuppressive agents (Table 1). Although there was no appreciable difference among the three group in total bilirubin values on admission, patients with FH had the highest mean aspartate aminotransferase values (3453 IU/l; $P = 0.0104$), the highest alanine aminotransferase values (4555 IU/l; $P = 0.0144$), and the lowest prothrombin activity (15.6%; $P < 0.0001$). However, there was a significant difference in the positivity for HBeAg among the three groups ($P = 0.0451$), and patients with FH had HBeAg on admission less frequently than those with AH (27.3% vs 72.7%; $P = 0.0240$). HBV genotype C predominated in each group (66.7%–81.8%), but there was no discernible difference in the frequency of genotypes/subgenotypes among the three groups. Ten (90.9%) of the 11 patients with FH were treated with plasma exchange, but no patients with AH or SAH received such treatment.

Quantitation of serum HBV DNA and serial changes in serum HBV DNA levels

In serum samples obtained within 24 h of admission, HBV DNA levels were 6.3 ± 1.8 log (copies/ml) in the AH group, 5.8 ± 1.2 log (copies/ml) in the SAH group, and 6.3 ± 1.6 log (copies/ml) in the FH group: the difference among the three groups was not statistically significant ($P = 0.6863$). Twenty-one patients who did not receive steroid pulse therapy, antiviral treatment, or plasma exchange (15 with AH and 6 with SAH) and 10 patients with FH who received plasma exchange were serially tested for serum HBV DNA levels (Fig. 1A–C). Serum HBV DNA level was highest on admission in 20 of the 21 patients. In patients with AH, serum HBV DNA level decreased gradually, and HBV DNA remained detectable even 30 days after the clinical onset (Fig. 1A). In patients with SAH, serum HBV DNA level tended to decrease rapidly and it could not be detected in 3 patients (50%) within 30 days from the clinical onset (Fig. 1B). Thus, the negativity rate for serum HBV DNA within 30 days after the clinical onset was significantly different in these two groups (0% vs 50%; $P = 0.0207$). In the 10 patients with FH who were treated with plasma exchange, the serum HBV DNA level decreased rather rapidly within 10 days from the clinical onset, irrespective of whether they had received steroid pulse, and the level further decreased gradually afterwards in 3 patients who recovered (Fig. 1C). The negativity rate of serum HBV DNA in the FH group was not compared with that in the other groups because 7 patients in the group died within 30 days from the disease onset. The HBV DNA level tended to decrease rapidly as the disease became more severe. The mean ± SD decrease of serum HBV DNA levels that were detected

Table 1. Clinical differences among patients with AH, SAH and FH

	AH ($n = 22$)	SAH ($n = 9$)	FH ($n = 11$)	P
Age (years)	37.2 ± 18.7	30.0 ± 7.8	39.5 ± 17.0	0.3909
Sex (M/F)	13/9	4/5	5/6	0.6584
Days between onset and admission	11.0 ± 7.9	7.0 ± 4.2	5.8 ± 2.5	0.0593
History of alcohol intake of over 50 g ethanol daily	4 (18.2%)	1 (11.1%)	1 (9.1%)	0.7033
Biological data on admission				
HBeAg status				
HBeAg-positive	16 (72.7%)	5 (55.6%)	3 (27.3%)	0.0451
Anti-HBe-positive	13 (59.0%)	5 (55.6%)	9 (81.8%)	0.3624
Total bilirubin (mg/dl)	6.4 ± 4.7	9.3 ± 7.2	8.6 ± 3.1	0.2640
AST (IU/l)	1301 ± 848	2342 ± 1776	3453 ± 3021	0.0104
ALT (IU/l)	2266 ± 1160	3197 ± 2045	4555 ± 3118	0.0144
Prothrombin time (%)	75.6 ± 24.6	37.1 ± 8.0	15.6 ± 10.2	<0.0001
Genotypes/Subgenotype				
A	2 (9.1%)	0	0	0.4226
Ba	1 (4.5%)	1 (11.1%)	0	0.6277
Bj	2 (9.1%)	1 (11.1%)	2 (18.2%)	0.7465
C	16 (72.7%)	6 (66.7%)	9 (81.8)	0.7350
H	0	1 (11.1%)	0	0.1529
Bj + C	1 (4.5%)	0	0	0.6277
Treatment				
Interferon	1 (4.5%)	0	0	0.6277
Lamivudine	3 (13.6%)	1 (11.1%)	1 (9.1%)	0.9271
Pulse steroid	2 (9.1%)	1 (11.1%)	1 (9.1%)	0.9834
Plasma exchange	0	0	10 (90.9%)	<0.0001

Values for continuous variables are expressed as means ± SD
AH, acute self-limited hepatitis; SAH, severe form of acute hepatitis; FH, fulminant hepatitis; HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase

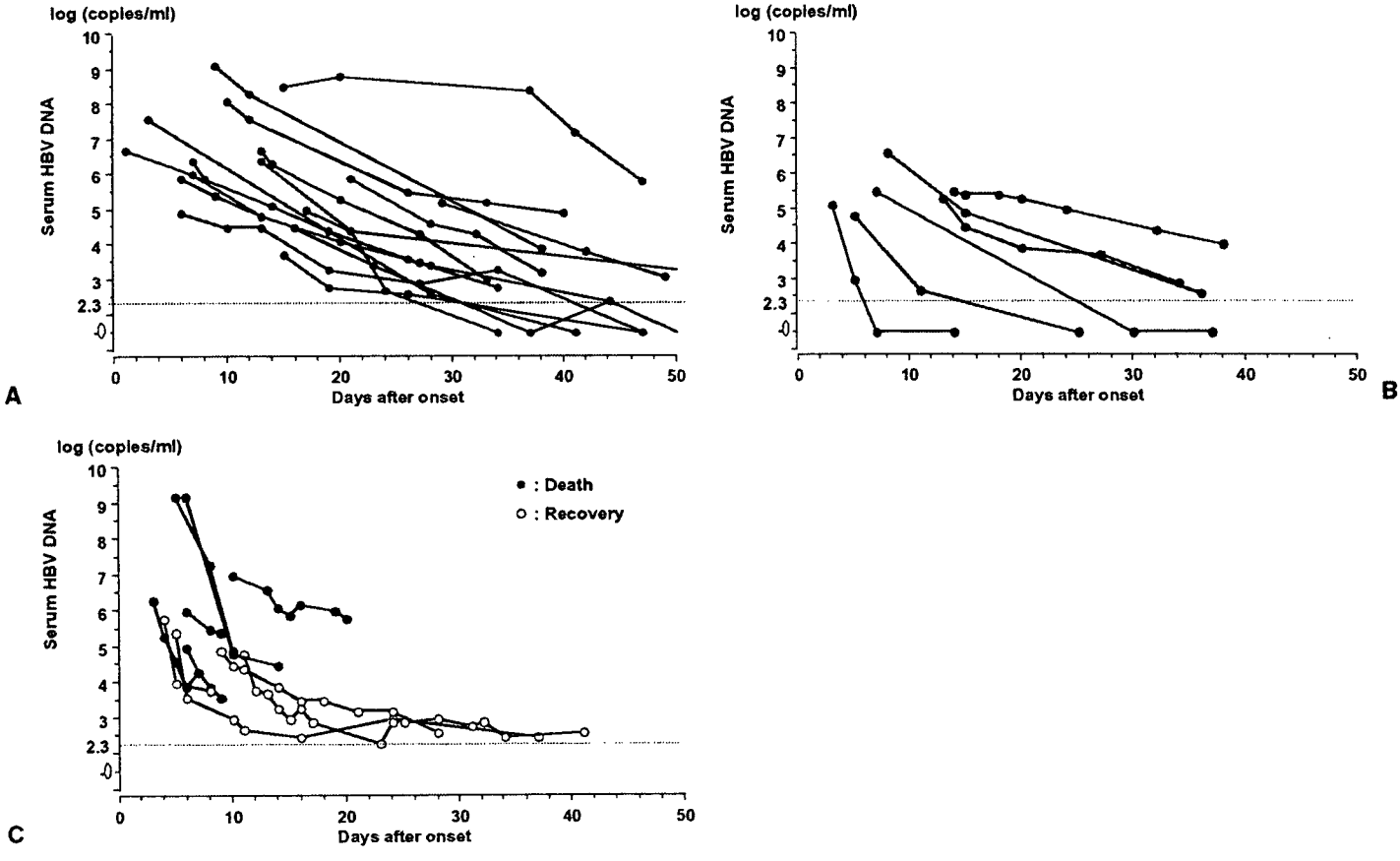

Fig. 1A–C. Serial changes in serum hepatitis virus B (*HBV*) DNA level in patients with **A** acute self-limited hepatitis, **B** severe form of acute hepatitis, and **C** fulminant hepatitis

at the first two time points within 10 days from the clinical onset was calculated to be 0.22 ± 0.19 log (copies/ml)/day in the AH patients (n = 4), 1.05 log (copies/ml)/day in the SAH patient (n = 1), and 0.97 ± 0.58 log (copies/ml)/day in the FH patients (n = 8), the difference being not statistically significant. Of note, the viremic level in the SAH/FH patients (n = 9) decreased significantly more rapidly than that in the AH patients (–0.98 ± 0.54 vs. –0.22 ± 0.19 log (copies/ml)/day; P = 0.0210).

Precore and core promoter mutations of HBV

HBV mutations in the precore and core promoter regions were G1896A (15 patients; 35.7%), G1899A (3 patients; 7.1%), A1762T/G1764A (14 patients; 33.3%), and T1753A/C and/or T1754C/G (14 patients; 33.3%). The frequency of mutations in the precore and core promoter regions was compared among the three groups (Fig. 2). G1896A was significantly more frequent in patients with FH than in those with AH (81.8% vs 9.1%; $P < 0.0001$) and SAH (81.8% vs 44.4%; $P < 0.05$). G1899A was less frequent than G1896A, and there was no significant difference in the frequency of G1899A among the three groups. The double mutation of A1762T/G1764A was significantly more frequent in patients with FH than in those with AH (81.8% vs 13.6%; $P < 0.0001$) and SAH (81.8% vs 22.2%; $P < 0.05$). Moreover, T1753A/C and/or T1754C/G occurred less frequently in patients with AH than in those with FH (13.6% vs 54.5%; $P < 0.05$) and SAH (13.6% vs 55.6%; $P < 0.05$). Mutations in both the precore (G1896A and/or G1899A) and core promoter regions (T1753A/C and/or T1754C/G and/or A1762T/G1764A) occurred more frequently in patients with FH than in those with AH (90.9% vs 9.1%; $P < 0.0001$) and SAH (90.9% vs 33.3%; $P < 0.05$).

Variables correlated with FH

To identify possible predictors of the development of FH among all patients studied, 13 potential variables were appropriately dichotomized, as listed in Table 2. Each of the mutations at nt 1753/1754, 1762/1764, 1896, and 1899 was regarded as a separate variable. Of these, seronegativity for HBeAg, G1896A, A1762T/G1764A, and mutations in both the precore and core promoter regions were significantly associated with FH (OR, 5.60; 95% confidence interval [CI], 1.22–25.8; P = 0.0269, OR, 18.8; 95% CI, 3.19–110; P = 0.0012, OR, 3.45; 95% CI, 0.82–14.5; P =0.0905, and OR, 52.0; 95% CI, 5.38–502; P = 0.0006, respectively). Mutations in both the precore and core promoter regions had the highest OR among the significant mutations. In multivariate logistic regression analysis, the only independent variable significantly associated with FH was the presence of mutations in both the precore and core promoter regions (OR, 42.8; 95% CI, 3.93–467; P = 0.0020).

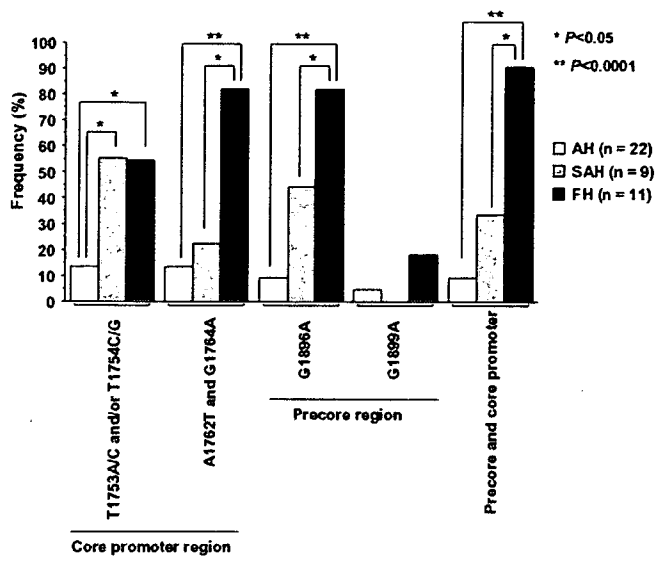

Fig. 2. Frequency of precore and core promoter mutations according to disease severity. *AH*, acute self-limited hepatitis; *SAH*, severe form of acute hepatitis; *FH*, fulminant hepatitis

Variables correlated with death

To assess variables correlated with death in patients with severe coagulopathy, we conducted a univariate analysis of 13 variables in 20 patients with SAH or FH (Table 3). Of these, only a serum HBV DNA level of more than 6.0 log (copies/ml) and mutations in both the precore and core promoter regions were significantly associated with a lack of recovery (OR, 13.8; 95% CI, 1.48–128; P = 0.0117 and OR, 5 820 000; 95% CI, 0-ND; P = 0.0048, respectively). In addition, there was no significant difference between patients who died and those who recovered in the duration from onset to admission (5.0 ± 1.7 vs 7.1 ± 3.8 days; P = 0.1883), but serum HBV DNA levels on admission were significantly higher in patients who died (7.0 ± 1.6 vs 5.6 ± 1.0 log [copies/ml]; P = 0.0293; Fig. 3). Two FH patients with more than 9.0 log (copies/ml) of serum HBV DNA were conscious on admission but died of encephalopathy, 13 and 14 days after admission. The multivariate regression analysis did not show significant variables associated with death, most likely due to the small number of cases studied.

Discussion

Several virological mechanisms may account for the development of FH in patients with acute HBV infection, but the involvement of HBV viral load in the early

Table 2. Univariate analysis of factors correlated with fulminant hepatitis B due to acute HBV infection

	Patients (*n*)	OR	95% CI	*P*
Age (years)				
>40	14	2.04	0.49–8.41	0.3253
≤40	28	1		
Sex				
Female	20	1.46	0.37–5.80	0.5399
Male	22	1		
Total bilirubin (mg/dl)				
>6.0	26	3.71	0.69–20.0	0.1282
≤6.0	16	1		
AST (IU/l)				
>2000	14	3.45	0.82–14.5	0.0905
≤2000	28	1		
ALT (IU/l)				
>3000	15	1.75	0.43–7.14	0.4352
≤3000	27	1		
HBeAg seropositivity				
Negative	18	5.60	1.22–25.8	0.0269
Positive	24	1		
HBV DNA (log copies/ml)				
>6.0	17	3.71	0.69–20.0	0.8395
≤6.0	25	1		
HBV genotype				
C	31	1.84	0.33–10.3	0.4861
Others	11	1		
Precore mutations				
G1896A				
Positive	15	18.8	3.19–110	0.0012
Negative	27	1		
G1899A				
Positive	3	6.67	0.54–82.3	0.0620
Negative	39			
Core promoter mutations				
T1753A/C and/or T1754C/G				
Positive	14	3.45	0.82–14.5	0.0905
Negative	28	1		
A1762T/G1764A				
Positive	14	23.4	3.84–143	0.0006
Negative	28	1		
Precore and core promoter mutations[a]				
Positive	15	52.0	5.38–502	0.0006
Negative	27	1		

HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase

[a] Precore and core promoter mutations; mutations in both the precore (G1896A and/or G1899A) and core promoter regions (T1753A/C and/or T1754C/G and/or A1762T/G1764A)

phase of FH has remained obscure. Recent articles reporting the dynamics of viral replication and liver injury in acute self-limited hepatitis B have shown that the onset of clinical symptoms of acute HBV infection coincides with the peak or initial decline in serum HBV DNA levels.[19,20] Our data on the serial changes in serum HBV DNA levels showed that, on admission, the majority of patients studied, had already passed the peak level of serum HBV DNA. However, the duration from onset to admission was not significantly different among the three groups in the study (AH, SAH, and FH). Therefore, comparison of the viral load on admission among the three groups in the present study seemed to be feasible. Although patients with FH received plasma exchange, steroid pulse, or antiviral therapy, the serum HBV DNA level tended to decrease rapidly within 10 days from the clinical onset as the disease became more severe. This finding may be ascribable to heightened immune responses in the early phase of SAH or FH.

The increased production of HBV in the liver and the resulting excessive presentation of HBV-related epitopes on the surface of hepatocytes to cytotoxic T cells may induce accelerated apoptosis. The hepatocytes that

Table 3. Univariate analysis of factors correlated with lack of recovery in patients with SAH and FH

	Patients (*n*)	OR	95% CI	*P*
Age (years)				
>40	6	7.33	0.87–61.3	0.0540
≤40	14	1		
Sex				
Female	11	1.14	0.18–7.28	0.8875
Male	9	1		
Total bilirubin (mg/dl)				
>8.0	10	4.00	0.55–29.1	0.1545
≤8.0	10	1		
AST (IU/l)				
>3000	8	3.00	0.45–20.2	0.2521
≤3000	12	1		
ALT (IU/l)				
>4000	10	1.20	0.19–7.72	0.8485
≤4000	10	1		
HBeAg seropositivity				
Positive	8	1.20	0.19–7.72	0.8485
Negative	12	1		
HBV DNA (log copies/ml)				
>6.0	7	13.8	1.48–128	0.0117
≤6.0	13	1		
HBV genotype				
C	15	2.67	0.24–30.1	0.4016
Others	5	1		
Precore mutations				
G1896A	13	5.14	0.48–55.7	0.1777
Positive	7	1		
Negative				
G1899A				
Positive	2	2.00	0.11–37.8	0.6440
Negative	18	1		
Core promoter mutations				
T1753A/C and/or T1754C/G				
Positive	11	1.14	0.18–7.28	0.8876
Negative	9	1		
A1762T/G1764A				
Positive	11	9.60	0.88–105	0.0640
Negative	9	1		
Precore and core promoter mutations[a]				
Positive	13	5820000	0-ND[b]	0.0048
Negative	7	1		

HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase

[a] Precore and core promoter mutations; mutations in both the precore (G1896A and/or G1899A) and core promoter regions (T1753A/C and/or T1754C/G and/or A1762T/G1764A)

[b] Upper limit data of 95% CI were not determined because 100% of patients with lack of recovery showed precore and core promoter mutation

are producing HBV diminish as the disease severity progresses. Therefore, the circulating HBV DNA level could be highest in AH if the replication activity of HBV is stable. However, to our surprise, the HBV DNA level at presentation did not differ significantly among the three groups in this study. It may be tempting to speculate that the production of HBV virions per hepatocytes in patients with FH may be heightened in patients with severe disease, although the destruction of hepatocytes proceeds swiftly in patients with FH. Therefore, in patients with SAH and FH, we evaluated variables that were correlated to death, including the serum HBV DNA level and the changing pattern of serum HBV DNA during the clinical course. To find factors affecting the clinical outcome in FH, we carried out univariate analysis in patients with SAH and FH who died and in those who recovered. Although mutations in both the precore and core promoter regions were significantly higher in nonsurvivors than in survivors, there was no difference in the frequency of those mutations, only a significant difference in viral load. Furthermore, all patients with severe coagulopathy and a high

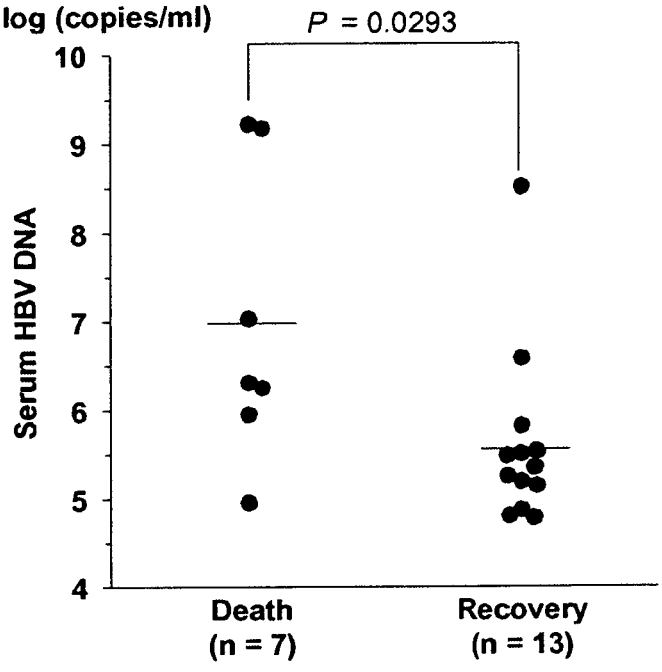

Fig. 3. Serum HBV DNA levels according to outcome among patients with severe form of acute self-limited hepatitis or fulminant hepatitis. The *horizontal bars* indicate the mean values

viral load, of more than 9.0log (copies/ml), died after being in a hepatic coma, regardless of the absence of encephalopathy on admission. Therefore, when a patient with acute hepatitis B develops severe coagulopathy, viral load is crucial for assessing the prognosis, and it may be beneficial to administer antiviral drugs in the initial phase within 10 days from the clinical onset.

Regarding the significance of the two precore mutations (G1896A and G1899A), it is known that they alter the regulation of HBV transcription and replication by providing a thermodynamically stable stem-loop structure (codons 28 and 29 in the precore region) that is involved in the encapsidation of HBV pregenomic RNA.[4,21,22] In this study, G1896A was predominant among precore mutations, and there were a few patients infected with HBV with the G1899A mutation, which was also accompanied by the G1896A mutation in one patient. These precore mutations in this study were closely correlated with the severity of disease, as described previously,[4,5] whereas there was no appreciable difference in HBV load on admission between patients with mutations in the precore region and those without (data not shown).

As for core promoter mutations, they are clustered within the region from positions 1750 to 1770 of the HBV genome, with the double mutations of A1762T and G1764A, being the most common.[23–30] Various mutations at nt 1753 and nt 1754, which confer the ability to secrete enveloped particles irrespective of the presence of core promoter mutations (A1762T/G1764A) and enhance HBV replication, were found more frequently in FH than in AH.[31,32] T1753A/C and/or T1754C/G mutations, which were accompanied by the double mutations of A1762T and G1764A in 57% of the patients in this study, occurred significantly more frequently in patients with FH than in those with AH or SAH. To date, most investigators have taken the approach of introducing the core promoter mutations into the background of a wild-type HBV genome and have focused on the double mutations of A1762T and G1764A.[33–35] When introduced into wild-type HBV genomes, the double mutations indeed decreased HBeAg expression level and also enhanced viral genome replication by two- to fivefold.[33,34] The decrease in HBeAg expression level is apparently mediated by a decrease in the precore mRNA transcription level, whereas the mechanism underlying enhanced replication may be more complex, involving both a transcription binding factor and the mutated HBx protein.[35] These findings suggest much-enhanced viral replication associated with FH.

Recently, Ozasa et al.[36] reported that patients with FH showed less positivity for HBeAg and were more frequently infected with Bj than those with AH, and that G1896A and A1762T/G1764A were more frequent in patients with FH than in those with AH. In our current study, although genotype C was predominant in each group and there was no significant difference in the distribution of genotypes/subgenotypes among the three groups, all FH patients with HBV genotype B were of subgenotype Bj. The close association of HBeAg negativity and the presence of G1896A and A1762T/G1764A with FH observed in the present study was confirmatory, compared with the previous study by Ozasa et al.[36] However, our present study revealed that the serum HBV DNA level decreased more rapidly in patients with more severe disease, and this was possibly caused by enhanced immune-mediated apoptotic liver damage. The high viral load in patients with severe coagulopathy increased the risk of mortality; therefore, intensive therapeutic strategies such as liver transplantation should be considered for such patients in the early phase of the disease.

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C型慢性肝炎に対するインターフェロンα-2b（イントロンA®），リバビリン併用療法の有用性

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