therapy [7, 8]. Some abrupt flares may be so severe that decompensation or even fulminant hepatic failure may occur [9–11]. Previous studies have identified pre-existing cirrhosis, high serum bilirubin levels, prolonged prothrombin time, pre-core/core promoter mutants, and high HBV DNA levels as factors associated with hepatic decompensation during AE in HBV carriers, though little is known about the predictive factors [9, 12, 13].

Liver transplantation is suitable therapy for acute hepatic failure, but the rate of liver transplantation has remained about 20% in Japan, where living donor liver transplantation is dominant [14, 15]. Thus, it is necessary to establish other effective therapies for patients with AE apart from liver transplantation. Steroids can rapidly inhibit excessive immune response and inflammatory reactions, and have been reported to be effective in cases of severe and potentially life-threatening exacerbation of chronic HBV (CHB) infection [16]. With the advent of oral nucleos(t)ide analogues (NAs), most guidelines recommend NAs for patients with AE of CHB infection [17–19], and several observational studies reported the use of NAs [9–11, 20, 21]. Timely use of potent anti-HBV agents, such as NAs, interferon (IFN), and steroids [22], during and/or after the development of hepatic decompensation could be potentially effective against various host- and virus-related factors.

The aim of the present study was to investigate the factor(s) that influence the rapid development of hepatic decompensation during AE of CHB.

Materials and Methods

Patients

The study subjects were patients with AE admitted to the Department of Hepatology, Toranomon Hospital, Tokyo, between 1984 and 2010. All patients were either followed up at our hospital with clinicopathologically proven CHB infection or were new patients with sudden-onset hepatic flares who visited our hospital outpatient clinic or were referred to our hospital from other clinics/hospitals. The diagnosis of CHB carrier state was established based on either positivity for hepatitis B surface antigen (HBsAg) for at least 6 months prior to the development of AE, or the presence of a high titer of anti-hepatitis B core antibodies (anti-HBcAb), together with negativity or a low titer of IgM anti-HBcAb. Chronic hepatitis and cirrhosis were confirmed by laparoscopy, needle biopsy, or ultrasonography, or treatment for these conditions for 1 year before the development of AE. AE of CHB infection was diagnosed by the following criteria: (1) an abrupt increase in serum alanine aminotransferase (ALT) levels to >300 IU/l in patients with original ALT levels of less than 5× the upper limit of normal or an abrupt two-fold increase in the serum ALT level to greater than 5× the upper limit of normal, (2) hyperbilirubinemia [serum bilirubin (Bil) >3.0 mg/dl], (3) evidence of coagulopathy with plasma prothrombin activity (PT) of <60% during the clinical course, and (4) lack of encephalopathy at admission. We also applied the following exclusion criteria: (1) the presence of viral markers other than HBV (hepatitis A, C, D, E, Epstein-Barr virus, cytomegalovirus, herpes simplex virus), (2) HBV reactivation induced by immunomodulators or chemo-/immunosuppressive therapy, (3) asymptomatic HBV carriers, (4) recent exposure to drugs and chemical agents as well as recent heavy alcohol intake, (5) breakthrough hepatitis caused by NAs, (6) evidence of decompensated liver disease before the onset of exacerbation as characterized previously, (7) HCC diagnosed by ultrasonography or computed tomography, and (8) coexistence of other serious medical conditions and other liver diseases, or metabolic diseases. Progression to severe acute exacerbation (SAE) was diagnosed by the development of hepatic encephalopathy of more than grade 2 within 8 weeks of onset associated with coagulopathy (PT <40%).

HBV DNA levels were measured serially to investigate the effects of HBV kinetics on the prognosis of patients with severe AE. HBV DNA levels were measured before treatment in 25 patients. "Before treatment" represented 1–8 weeks before commencement of treatment. HBV DNA levels were also measured after treatment in 27 patients. "After treatment" was defined as 2 weeks after commencement of therapy. Viral kinetics was assessed using the same assay in all individuals. The Local Ethics Committee of Toranomon Hospital approved the study, and informed consent was obtained from all patients.

Virological markers

Serial blood samples were obtained during the clinical course of AE and stored at −80°C until used for HBV molecular analysis. Serological tests for HBsAg, HBsAb, hepatitis e antigen (HBeAg), IgM anti-HBcAb, total anti-HBcAb, and anti-HBeAb were conducted using radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA) according to the instructions provided by the manufacturer. Precore (PC) mutations were analyzed by PCR enzyme-linked mini-sequence assay (Roche Diagnostics, Tokyo, Japan), and basal core promoter (BCP) mutations were analyzed by PCR specific probe assay (Roche Diagnostics, Tokyo, Japan). HBV DNA was measured by Amplicor monitor assay (dynamic range 2.6–7.6 log copies/ml, Roche Diagnostics, Tokyo, Japan), COBAS TaqMan v.2.0 (dynamic range 2.1–9.0 log copies/ml, Roche Diagnostics), transcription-mediated amplification and hybridization

protect assay (TMA-HPA) (dynamic range 3.7–8.7 LGE/ml, Chugai Diagnostics Science Co., Tokyo) or sandwich hybridization assay with signal amplification using branched DNA (bDNA, dynamic range 0.7–3800 Meq/ml). The major genotype of HBV was determined using enzyme-linked immunosorbent assay (ELISA, Institute of Immunology, Tokyo, Japan) or PCR-invader assay (BML, Inc, Tokyo, Japan) based on the methods described previously [23, 24]. HBVDNA levels assessed by bDNA were re-measured by TaqMan PCR assay using stored serum samples.

Statistical analysis

Continuous variables were expressed as median (range), and compared by Mann–Whitney *U* test. Categorical variables were compared by χ^2 test or Fisher's exact test, as appropriate. Univariate analysis was applied to determine the relationship between SAE and each of the following factors: sex, age, presence of compensated cirrhosis, and various biological and virological markers as measured at baseline (bilirubin, PT, ALT, albumin, HBeAg, HBV DNA, and HBV genotype, PC and BCP mutations). Each continuous variable was transformed into two categories based on the value with the largest capacity to discriminate between patients for univariate and multivariate analyses. Factors that correlated significantly with SAE were entered into multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence intervals (95% CI) were determined. All analyses were performed using The Statistical Package for Social Sciences (SPSS II v. 11.0, Chicago, IL, USA), and statistical significance was taken as a two-sided P value <0.05.

Results

Clinical features of severe acute exacerbation

A total of 37 patients (30 men and 7 women) fulfilled the criteria of AE and were included in this study. The baseline characteristics at the commencement of therapy of these 37 patients are shown in Table 1. Twenty-two patients were observed at our hospital, and 15 patients were referred from another hospital after the onset of hepatic flares. The majority of patients had genotype C, and 27 patients (72.9%) were HBeAg positive. The PC and BCP mutations were determined in 27 patients; 22 patients had mutations in the PC region, 16 patients had mutations in the BCP region, and 12 patients had mutations in both the PC and BCP regions. During the clinical course, the peak median values were: ALT 713 IU/l (range 307–2857), bilirubin 8.4 mg/dl (3.0–51.4), and PT 47.6% (12.0–60.0).

Table 1 Baseline characteristics of the 37 patients infected with HBV who developed severe acute exacerbation at the commencement of therapy

Number	37
Sex (male/female)	30/7
Age (years)	45 (23–63)
Family history (yes/no)	21/16
Cirrhosis (present/absent)	7/30
Albumin (g/dl)	3.4 (2.5–4.6)
Bilirubin (mg/dl)	4.7 (1.0–30.7)
AST (IU/l)	601 (64–2593)
ALT (IU/l)	657 (124–2142)
LDH (IU/l)	297 (106–594)
Platelets ($\times 10^4$/mm^3)	12.3 (6.2–32.0)
α-Fetoprotein (μg/ml)	62.0 (3.0–1600)
Prothrombin activity (%)	53 (26–80)
Genotype (A/B/C)	0/5/32
HBeAg (positive/negative)	27/10
HBV-DNA ($\log_{10}$ copies/ml)	8.5 (6.8–8.9)
PC (wild/mutant/ND)	5/22/10
BCP (wild/mutant/ND)	11/16/10

Data are median values (range) or number of patients

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *HBeAg* hepatitis B envelope antigen, *PC* pre core, *BCP* basal core promoter, *ND* not done

Treatment

NAs were used in 19 patients, IFN in 8, and corticosteroids (CS) in 20 patients. In addition, 7 patients were treated with a combination of NAs and CS; 2 patients were treated with three drugs (NAs, IFN, and CS). At the time of the study, lamivudine (LMV) was not yet available for the treatment of chronic hepatitis B, and thus IFN was used; 6 patients were treated with both IFN and CS. None of the patients underwent liver transplantation.

Prognosis of severe acute exacerbation and factors associated with progression to hepatic failure

Of the 37 patients admitted with CHB infection and AE, 23 (62.2%) did not develop SAE. The remaining 14 (37.8%) patients developed SAE; 9 (24.3%) patients died of liver-related death, but 5 (13.5%) survived. Further analysis showed that 8 (36.4%) of 22 patients who were observed in our hospital developed AE, and 6 (27.3%) of these patients died, whereas 6 (40.0%) of 15 patients who were referred from other hospitals after the onset of exacerbation developed AE, and 3 (20.0%) of these patients died. There was no significant difference in prognosis by treatment facility before AE. Ten of 37 patients experienced AE before 2000 when LMV was available in Japan, and 19

Table 2 Biochemical, virological and histological features of patients with severe acute exacerbation at the commencement of therapy

Case	Age (years)/ sex	Genotype	HBeAg	HBV-DNA (log copies/ ml)	Preexisting cirrhosis	Serum bilirubin (mg/dl)	ALT (IU/l)	PT (%)	Platelets ($\times 10^4$/ mm^3)	Therapy	Outcome (time from treatment to death, weeks)
1	63/M	B	–	8.4	No	5.8	1680	43	6.2	LMV + CS	Death (11)
2	32/M	B	–	>8.7	No	6.9	1340	41	13.4	CS	Death (1)
3	58/M	B	–	8.6	No	7.4	1446	36	7.7	CS	Death (2)
4	29/M	B	–	>8.7	No	15.6	307	26	10.0	LMV	Recovery (alive)
5	54/F	C	+	>8.7	No	2.4	2077	79	21.0	LMV + CS	Recovery (alive)
6	37/M	C	+	>8.7	No	4.1	552	53	8.9	CS	Recovery (alive)
7	62/M	C	+	7.0	No	12.0	220	53	7.1	LMV + CS + IFN	Recovery (alive)
8	33/F	C	+	>8.7	No	14.0	632	39	13.1	CS	Recovery (alive)
9	55/M	C	+	>8.7	Yes	4.0	1089	55	10.3	LMV + CS	Death (1)
10	37/F	C	+	7.1	Yes	5.8	1444	34	22.0	LMV + CS + IFN	Death (10)
11	49/M	C	+	8.0	Yes	8.8	834	58	9.9	CS	Death (10)
12	33/M	C	+	8.5	No	9.6	657	26	7.4	LMV + CS	Death (2)
13	54/M	C	+	7.8	Yes	12.1	364	36	15.8	LMV + CS	Death (2)
14	55/M	C	+	>8.7	No	24.2	520	44	8.3	CS	Death (5)

Abbreviations as in Table 1, *PT* prothrombin activity, *LMV* lamivudine, *CS* corticosteroids, *IFN* interferon-α

patients experienced AE after 2000. The other 8 patients experienced AE before 2000, but received LMV through participation in clinical trials or paid for the drug privately. The clinical features at the commencement of therapy of 14 patients who developed SAE are shown in Table 2 (median age 52 years, range 29–63). The mean time period between admission and death of 9 patients who developed SAE was 2 (range 1–11) weeks. Six patients who were admitted before the availability of LMV were treated with CS alone, 5 patients were treated with the combination of LMV and CS, 1 patient was treated with LMV alone, and 2 other patients were treated with LMV, CS, and IFN. Among 8 patients treated with LMV, of those who developed SAE, 5 died, and 2 patients developed complications caused by bacterial infection. Four patients had genotype B, while 10 patients had genotype C. HBeAg status was positive in 10 patients. The mean HBV DNA level was 8.7 (range 7.0–>8.7) log copies/ml, ALT 746 (220–2077) IU/l, serum bilirubin 8.1 (2.4–24.2) mg/dl, PT 42 (26–79)%, and platelet count was 10.0 (62–220) $\times$ $10^4/mm^3$.

Of the 5 patients who were treated successfully after progression to SAE, one later died of severe breakthrough hepatitis caused by emergence of LMV-resistant virus 3 years after SAE (case 7, Table 2). The other four survived (cases 4–6 and 8, Table 2).

Table 3 shows the results of univariate analysis. The following factors showed significant relationship with the development of SAE at the commencement of treatment: serum bilirubin (>5 mg/dl) and PT (<60%). Multivariate analysis identified serum bilirubin as a significant and independent determinant of the development of SAE (Table 3). On the other hand, two parameters showed significant relationships with liver-related death: serum bilirubin (>7 mg/dl, $P = 0.049$) and PT (<45%, $P = 0.003$). Multivariate analysis identified PT (OR 9.50, 95% CI 1.3–71.0, $P = 0.028$) as a significant determinant of death.

Viral kinetics associated with fulminant hepatic failure

To investigate the relationship between viral kinetics and SAE, HBV DNA levels were measured in 25 patients both before and commencement of treatment and also after treatment in 27 patients. Figure 1 shows the viral load of patients who developed and did not develop SAE at commencement of treatment compared with before treatment. Falls in the HBV DNA level occurred naturally. However, in 11 patients who developed SAE, HBV DNA levels increased in 6 patients and did not change in 5 patients. Among the latter 5, HBV DNA levels of 4 patients were >8.7 log copies/ml. In 14 patients who did not develop SAE, HBV DNA levels increased in 4 patients, were unchanged in 4 patients, and decreased in 6 patients. Hence, the HBV DNA level increased/was unchanged in 8 of 14 (57%) patients who did not develop SAE, compared with 11 of 11 (100%) patients who developed SAE. A significantly higher proportion of patients with SAE showed an increase/was unchanged in viral load compared to those who without SAE ($P = 0.02$). We also examined the viral kinetics in 27 patients by comparing HBV DNA levels at the commencement of treatment to after treatment.

Table 3 Univariate and multivariate analyses of host and viral factors associated with progression of severe acute exacerbation at commencement of treatment

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex (female)	1.30 (0.15–4.11)	0.76		
Age (>55 years)	2.64 (0.57–12.3)	0.22		
Cirrhosis (present)	1.90 (0.39–9.26)	0.43		
Albumin (<3.5 g/dl)	1.75 (0.44–6.97)	0.85		
Bilirubin (>5 g/dl)	17.0 (2.92–99.1)	0.002	11.2 (1.71–73.8)	0.01
ALT (>800 IU/l)	1.88 (0.48–7.26)	0.36		
AST/ALT ratio (>1)	1.27 (0.31–5.19)	0.74		
Prothrombin activity (<60%)	11.9 (1.33–106.7)	0.03	8.22 (0.73–92.6)	0.09
Platelets (<15 × 10^4/mm^3)	0.81 (0.19–3.58)	0.89		
Genotype (B)	8.82 (0.87–89.1)	0.06		
HBeAg (positive)	0.89 (0.20–3.90)	0.89		
HBV-DNA (>8.7 log copies/ml)	2.34 (0.60–9.20)	0.70		
PC mutation	2.29 (0.22–24.1)	0.49		
BCP mutation	0.19 (0.034–1.08)	0.06		

Abbreviations as in Tables 1 and 2, *OR* odds ratio, *CI* confidence level

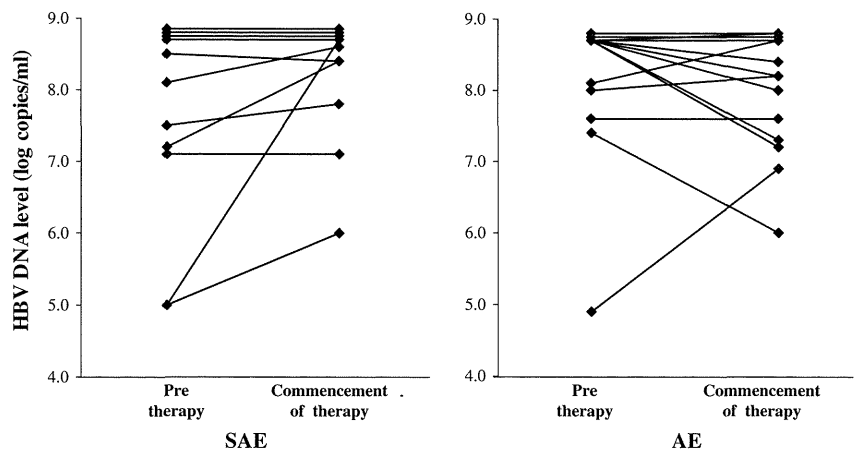

Fig. 1 Viral kinetics from pre-treatment to commencement of treatment in patients with acute exacerbation. Viral kinetics tended to increase or remained unchanged until treatment in 8 patients with acute exacerbation course ($n = 14$), while the viral load in all patients with severe acute exacerbation ($n = 11$) increased or remained unchanged ($P = 0.02$)

The HBV DNA level decreased more than 1 log copies/ml in 9 of 17 (52.9%) patients who did not develop SAE, compared with 3 of 10 (30.0%) patients who developed SAE, but the difference between the two groups was not significant.

Discussion

The results of the present study examined the predicting factors of progression to SAE accompanied by coagulopathy and encephalopathy in patients with AE of chronic hepatitis B, as well as the pattern of viral kinetics before and after commencement of therapy. Up to 30% of patients with CHB infection experience reactivation of hepatitis every year [5, 6], while some patients develop acute exacerbation with jaundice and coagulopathy, a severe life-threatening condition with high mortality [9, 12]. It is important to determine the predicting factors of progression to liver decompensation in patients with acute exacerbation. Multivariate analyses in previous studies indicated that pre-existing cirrhosis, a high Child–Pugh score, low albumin level, high serum bilirubin level, prolonged PT, and high HBV DNA levels were associated with the severity or mortality during acute exacerbation [9, 12, 13]. Our results are almost comparable to those of the above studies. Multivariate analysis in the present study identified the serum bilirubin level as a predictor of progression to liver decompensation. Moreover, there were no significant differences in viral load or therapeutic regimen. Genotype B was the predominant HBV strain in patients with SAE compared to patients with variable severity of liver diseases [25]. The frequencies of HBV genotype in patients with chronic hepatitis B admitted to our hospital were 3.0, 12.3, and 84.5%, for genotypes A, B, and C, respectively [26]. In the present study, although patients

with genotype B were only 5 of the total 37 (13.5%), 4 of 14 (28.6%) patients with SAE and 3 of 9 (33.3%) patients who died of liver failure were infected with genotype B. The different HBV genotypes also cause different clinical and epidemiological features. In a study from Japan, a high prevalence of genotype B HBV was found among patients with acute fulminant hepatitis [27]. In two case control studies conducted in Hong Kong, genotype B was the predominant HBV strain among patients with SAE compared to control patients with various severities of liver diseases [25, 28]. In this regard, another study indicated that genotype Bj was associated with high extracellular expression of HBV DNA in vitro [29]. The tendency of genotype Bj to produce high extracellular virion levels would be associated with a more vigorous immune response, leading to a higher risk of hepatic decompensation during the hepatitis flare. Several studies examined the association between specific mutations in the HBV genome and fulminant hepatitis or acute-on-chronic liver failure, especially in the PC (nt 1896) and BCP (nt 1762 and 1764) regions [30–32]. The PC and BCP regions are crucial replications of HBV [33], so alteration of the phenotype by the emergence of mutations in the PC and BCP regions might causes changes in the relationship between the virus and hepatocytes [30], and lead to fulminant hepatitis and acute exacerbation of chronic hepatitis. In the present study, genotype B and PC/BCP mutations were not significant predictors associated with the development of SAE or liver-related death, which is probably related to the small number of cases.

Jeng et al. [13] reported that HBV DNA levels greater than 1.55×10^9 copies/ml in patients with AE may predict subsequent occurrence of hepatic decompensation. While the overall viral load in our subjects was high (8.5 log copies/ml, Table 1), there was no relationship between viral load and the severity of AE or mortality. In addition, the HBV DNA level could not be estimated correctly when it was above the upper limit. Interestingly, the level of HBV DNA re-measured by TaqMan PCR in stored blood samples was higher than the upper limit (>9.1 log copies/ml) in one-third of the patients. The extremely high HBV DNA levels in patients with AE suggest that the vigorous immune attack on HBV and resultant liver injury will continue and may progress into hepatic decompensation. The present results showed that the decrease of viral load was significantly lower in patients with fulminant hepatic failure than in those with AE. These findings suggest that viral kinetics before the commencement of therapy are an important predictor of hepatic decompensation in patients with CHB infection complicated with AE. Interestingly, there was no significant difference in viral kinetics after the commencement of therapy between the two groups. To our knowledge, this is the first report that identifies viral kinetics before the commencement of therapy as a predictor of prognosis of patients with AE of chronic hepatitis B.

LMV monotherapy does not seem to improve short-term mortality in patients with AE [9], although other studies showed a possible decrease in the mortality rate with earlier administration [21]. In a recent randomized trial designed for the treatment of acute-on-chronic liver failure due to severe reactivation of hepatitis B, the use of tenofovir significantly reduced the mortality rate compared with placebo [11], and the results suggested that rapid suppression of HBV DNA replication with potent antiviral therapy could inhibit the ongoing necroinflammation and permitted hepatic regeneration. Although 8 of 14 patients were treated with LMV in the present study, two patients had to start LMV after the development of SAE because of the rapid exacerbation soon after admission. Five patients developed SAE within a median period of 8 days (range 1–17 days) after the commencement of LMV. The other one patient developed complications caused by bacterial infection and gradually progressed to liver failure over 2 months. Thus, it is thought that most of these patients developed SAE earlier than the available effect of LMV.

The prevailing idea is that AE is the result of a robust quantitative recovery of HBV specific T cells, which directly cause liver injury [34]. Other mechanisms of the effects of CS in AE may be related to the prevention of endotoxin-induced secondary liver injury [35], prevention of cytolysis of ballooned hepatocytes by stabilization of the lysosomal membrane [36], and improvement of the functional activity of the remaining hepatocytes [37]. Other studies showed that the preferential increase in the number of HBV-specific CD8 T and CD4 T cells is associated with viral control rather than liver damage [38, 39]. Whatever the mechanism of AE, a few weeks are needed for sufficient suppression of the production of HBV-related proteins by preventing HBV replication even when NAs are used [40]. Thus, earlier introduction of CS in combination with potent antiviral therapy is a reasonable approach for the initial treatment of AE to prevent excessive immunological reactions and progression of liver cell injury [22, 41]. NA or CS used on its own has limits in the resolution of the serious conditions. Considered together, it is necessary to establish effective standardized strategies, such as the combination of NA and CS. Moreover, to provide cover for NA, especially for the time until NA starts to exert its potent antiviral effect, IFN could be added with NA and CS.

In conclusion, the results of this study suggest that viral kinetics before therapy may influence the clinical course and fate of patients with SAE complicating chronic hepatitis B. Antiviral therapies, including NA and/or IFN with CS, should be started as soon as possible in cases with high serum bilirubin and/or low PT levels, genotype B, and viral

load to prevent progression into hepatic decompensation. Although ethical issues could be an obstacle to randomized trials in such severe cases, more effective strategies are necessary for the treatment of AE associated with chronic hepatitis B.

Acknowledgment This study was supported in part by a Grant-in-aid from the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest The authors declare no conflict of interest.

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Hepatology Research

Hepatology Research 2012; 42: 558–563 doi: 10.1111/j.1872-034X.2011.00957.x

Original Article

Prevalence and predictive factors of diabetes in hepatitis virus positive liver cirrhosis with fasting plasma glucose level of <126 mg/dL

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Aim: The aim of this study was to evaluate the prevalence and predictive factors of diabetes in hepatitis virus positive liver cirrhotic patients with fasting plasma glucose (FPG) level of <126 mg/dL.

Methods: A total of 263 patients with hepatitis C virus (HCV) or hepatitis B virus (HBV) positive liver cirrhosis, FPG level of <126 mg/dL, and had diabetes status evaluated by the use of 75-g oral glucose tolerance test (OGTT), were enrolled in this study. Plasma glucose and insulin levels were analyzed periodically for 3 h after oral glucose loading. Diabetes was defined as a 2-h post-load glucose on the OGTT of ≥200 mg/dL. The prevalence of diabetes by use of OGTT and predictive factors for diabetes were evaluated by the use of the Mann–Whitney *U*-test, Fisher's exact probability test or multivariate analysis by logistic regression. Hypoalbuminemia was defined as serum albumin level of <3.9 g/dL. Elevated indocyanine green retention rate at 15 min (ICG $_R$15) was regarded as ≥ 25%.

Results: Out of 263 patients, 44 (16.7%) were diagnosed as having diabetes. Multivariate analysis showed that diabetes occurred when patients had hypoalbuminemia of <3.9 g/dL (odds ratio [OR] 2.33; 95% confidential interval [CI] = 1.04–5.24; $P = 0.040$) and ICG $_R$15 of <25% (OR 2.36; 95%CI = 1.01–5.58).

Conclusions: Hypoalbuminemia and elevated ICG $_R$15 in hepatitis virus related cirrhotic patients with FPG level of <126 mg/day enhance diabetes pattern after OGTT with significant difference.

Key words: diabetes mellitus, hepatitis virus, liver cirrhosis, oral glucose tolerance test

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the more common causes of chronic liver disease worldwide. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis and/or hepatocellular carcinoma over a period of 10–30 years.[1–8] Lately, it have been reported that chronic HCV infection is associated with type 2 diabetes mellitus (T2DM).[9–11] Moreover, T2DM has been suggested to enhance with the development of HCC and poor prognosis of liver transplantation.[12–15] Thus, early intervention to prevent or improve T2DM is necessary to get good prognosis in HCV patients.

However, the big problem in chronic liver disease is that fasting serum glucose (FPG) often shows normal level. Hence, examination of oral glucose tolerance test (OGTT) is necessary to evaluate diagnosis of precise diabetes in patients with chronic liver disease. With this background, we evaluated the prevalence of abnormal glucose state and predictive factors for diabetes in HCV positive liver cirrhosis patients with fasting plasma glucose level of <126 mg/dL. We investigated the

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Received 4 September 2011; revision 7 November 2011; accepted 2 December 2011.

prevalence of abnormal glucose state and predictive factors for diabetes in hepatitis B virus (HBV) positive patients with liver cirrhosis and compared with HCV.

METHODS

Patients

A TOTAL OF 263 Japanese patients who were diagnosed with liver cirrhosis by laparoscopic and/or histological findings from December 1998 to January 2005 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan were enrolled. Inclusion criteria were as follows: (i) evidence of liver cirrhosis by laparoscopy and/or histological findings; (ii) FPG level of <126 mg/dL; (iii) evidence of HCV or HBV by serum examination; (iv) negativity for antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by indirect immunofluorescence assay; (v) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; (vi) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Patients with any of the following criteria were excluded from the study: (i) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites, (ii) a history of diabetes, (iii) taking medicines that may influence on glucose tolerance such as branched chain amino acid (BCAA), thiazide diuretics, and angiotensin receptor antagonist.

A total of 263 patients with FPG of <126 mg/dL undertook a 75-g OGTT. Plasma glucose levels were analyzed periodically for 3 h after oral glucose loading. Impaired glucose tolerance (IGT) were defined as a 2-h post-load glucose on the OGTT of ≥140 mg/dL, but <200 mg/dL. Diabetes was defined as a 2-h post-load glucose on the OGTT of ≥200 mg/dL. T2DM was diagnosed by the use of the 2003 criteria of the American Diabetes Association.[16]

The index of insulin resistance was calculated on the fasting glucose and insulin by the homeostasis model for insulin resistance (HOMA-IR). Insulin secretion was calculated by the insulinogenic index (IGI); IGI = (Ins30-Ins0)/(Glc30-Glc0), Ins0: fasting plasma insulin (mU/L); Ins_{30}: insulin 30 min after glucose intake (IU/mL); Glc0: fasting plasma glucose (mg/dL); and Glc30; plasma glucose 30 min after glucose intake (mg/dL).

The physicians in charge explained the purpose and method of the OGTT to each patient. Informed consent was obtained from all patients included in the present study. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Clinical and laboratory analysis

Anthropometric analysis included height, weight and body mass index (BMI), and the latter was calculated as weight (kg) divided by the square of the height (m^2). Laboratory analysis was performed via standard laboratory methods. The biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride, albumin, platelet, fasting plasma glucose (FPG) and fasting insulin. Serum insulin levels were measured with a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Hypoalbuminemia was defined as serum albumin level of <3.9 g/dL. Elevated indocyanine green retention rate at 15 min (ICG_R15) was regarded as ≥25%.

Laboratory investigation

Hepatitis B surface antigen (HBsAg) was assayed using commercially available radioimmunoassay kits. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay. HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche, Tokyo, Japan). HbA1c was measured using a high performance liquid chromatography (HPLC) method. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg)/height (in m^2).

Statistical analysis

The results are presented as means ± standard deviation (SD) or as numbers. Statistical differences in quantitative data were determined using the Mann–Whitney *U*-test, Fisher's exact probability test or multivariate analysis by logistic regression.

Multivariate analysis for diabetes was carried out by logistic regression. The Statistical Program for Social Sciences software package (SPSS 11.5 for Windows, SPSS, Chicago, IL, USA) was used to perform statistical analysis. A P-value < 0.05 was considered to be statistically significant.

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics at the day of evaluating OGTT in the 263 enrolled patients. The

Table 1 Clinical characteristics of cirrhotic patients with hepatitis C virus (HCV)†

Characteristic	
n	263
Sex (male/female)	178/85
Age (years)	51.6 ± 11.2
Body mass index	21.8 ± 3.0
HBV/HCV	96/167
Fasting plasma glucose (mg/dL)	84 ± 13
Albumin (g/dL)	4.1 ± 0.5
Total cholesterol (g/dL)	163 ± 37
HDL cholesterol (g/dL)	47 ± 13
Triglyceride (mg/dL)	98 ± 35
Uric acid (mg/dL)	5.2 ± 1.2
AST (IU/L)	78 ± 70
ALT (IU/L)	72 ± 68
GGT (IU/L)	74 ± 50
Platelet (×104/mm^3)	11.3 ± 4.1
ICG_R15 (%)	25.4 ± 14.0

†Data are number of patients or mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HBV, hepatitis B virus; HDL, high density lipoprotein; ICG_R15; indocyanine green retention rate at 15 min.

mean age was 51.6 years and mean FPG level was 84 mg/dL. The serum albumin level was 4.1 ± 0.5 g/dL and ICG_R15 was 26.5 ± 14.0%. On the diagnosis of liver cirrhosis, 160 of 263 patients were diagnosed by laparoscopy and liver biopsy; 69 patients were diagnosed by laparoscopy only; 34 patients were diagnosed by biopsy only.

Prevalence of IGT and diabetes in hepatitis virus positive liver cirrhosis with FPG of <126 mg/dL

Out of 263 patients who had hepatitis virus-related liver cirrhosis with FPG of <126 mg/dL, 44 (16.7%) patients were diagnosed as having DM and 73 (27.8%) patients were diagnosed as having IGT. Table 2 shows the predictive factors for DM pattern by the use of OGTT in hepatitis virus related cirrhotic patients. Multivariate analysis showed that diabetes occurred when patients had hypoalbuminemia of <3.9 g/dL (odds ratio [OR] 2.33; 95% confidential interval [CI] = 1.04–5.24; $P = 0.040$) and ICG_R15 of <25% (OR 2.36; 95%CI = 1.01–5.58). Table 3 shows the incidence of diabetes based on serum albumin and ICG_R15. The incidence of diabetes in patients with hepatitis virus related liver cirrhosis was 5.8% (7/120) in group A with serum albumin level of ≥3.9 g/dL and ICG_R15 of <25%. On the other hand, that was 35.6% (21/59) in group B with hypoalbuminemia of <3.9 g/dL and ICG_R15 of ≥25%.

Changes of glucose state based on difference of serum albumin level

Table 4 shows the glucose and insulin dynamics after OGTT in cirrhotic patients that belonged to group A with serum albumin level of ≥3.9 g/dL and ICG_R15 of

Table 2 Predictive factors for diabetes in cirrhotic patients with hepatitis C virus (HCV)

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (per 10 years)	1.50 (1.04–2.16)	0.031		
Gender (M/F)	1.08 (0.52–2.23)	0.842		
Body mass index (per 5)	1.18 (0.59–2.36)	0.631		
HCV/HBV	2.38 (1.05–5.43)	0.039		
AST (IU/L, ≥37/<37)	1.01 (0.45–2.25)	0.996		
ALT (IU/L, ≥42/<42)	0.81 (0.40–1.65)	0.563		
GGT (IU/L, ≥109/<109)	1.89 (0.66–5.45)	0.238		
Platelet (×10^4/mm^3, <10/≥10)	2.59 (1.26–5.32)	0.009		
Albumin (g/dL, <3.9/≥3.9)	3.40 (1.66–6.94)	0.001	2.33 (1.04–5.24)	0.040
Triglyceride (mg/dL, ≥150/<150)	2.26 (0.74–6.90)	0.152		
Total cholesterol (mg/dL, ≥180/<180)	0.69 (0.30–1.60)	0.387		
HDL cholesterol (mg/dL, <40/≥40)	1.09 (0.43–2.73)	0.857		
ICGR15 (%, ≥25/<25)	3.64 (1.67–7.95)	0.001	2.36 (1.01–5.58)	0.049

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HBV, hepatitis B virus; HDL, high density lipoprotein; ICG_R15, indocyanine green retention rate at 15 min; OR, odds ratio.

Table 3 Diabetic rate based on serum albumin and ICG $_R$15

	Albumin; ≥3.9 g/dL	Albumin; <3.9 g/dL	Total
ICG $_R$15 < 25 (%)	5.8% (7/120)	15.0% (3/20)	7.1% (10/140)
ICG $_R$15 ≥ 25 (%)	20.3% (13/64)	35.6% (21/59)	27.6% (34/123)
Total	10.9% (20/184)	30.4% (24/79)	16.7% (44/263)

ICG$_R$15, indocyanine green retention rate at 15 min.

<25% or group B with serum albumin level of <3.9 g/dL and ICG $_R$15 of ≥25%. The serum glucose levels at 0, 60, 90, 120, and 180 min after the initiation of OGTT in patients with serum albumin level of <3.9 g/dL and ICG $_R$15 of ≥25% were statistically higher than those in patients with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of <25%. HOMA-IR in patients with serum albumin level of <3.9 g/dL and ICG $_R$15 of ≥25% was higher than that in patients with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of <25%. IGI in patients with serum albumin level of <3.9 g/dL and ICG $_R$15 of ≥25% was lower than that in patients with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of <25%.

DISCUSSION

WE HAVE DESCRIBED the prevalence of abnormal glucose state and predictive factors for diabetes in HCV or HBV positive liver cirrhosis patients with fasting plasma glucose level of <126 mg/dL in the present study. Enrolled patients had liver cirrhosis diagnosed with laparoscopy and/or histological examination. There are sometimes discrepancies between laparoscopic finding and histological findings in patients with HCV.[17] Thus, in the present study, cirrhotic patients diagnosed by either laparoscopy and/or histological examination were enrolled.

The present study shows several findings with regard to the prevalence of abnormal glucose state in hepatitis virus related cirrhotic patients with FPG level of <126 mg/dL. First, approximately 17% of the cirrhotic patients with FPG level of <126 mg/dL had diabetic pattern by the OGTT. If OGTT was not performed in patients who were diagnosed as having diabetes after OGTT, diabetes would be missed.

Second, multivariate analysis suggested that lower serum albumin level and elevated ICG$_R$15 were independent risk factors of diabetes mellitus. Our result shows that patients with hypoalbuminemia and elevated ICG$_R$15 should pay attention to complication of T2DM even if FPG is in the normal range. In the present study, hypoalbuminemia was defined as serum albumin level of <3.9 g/dL and elevated ICG $_R$15 was regarded as ≥25%. As the serum albumin level (mean ± standard deviation)

Table 4 Glucose and Insulin dynamics after oral glucose tolerance test (OGTT) in cirrhotic patients

	Group A (albumin; <3.9 g/dL) (ICG $_R$15; ≥25%)	Group B (albumin; ≥3.9 g/dL) (ICG $_R$15; <25%)	*P*-value
Number	59	120	
HOMA-IR	3.22 ± 2.24	2.14 ± 1.12	0.003
IGI	0.70 ± 0.53	0.96 ± 0.83	0.042
Glucose (mg/dL)			
At 0 min	89.3 ± 12.0	82.8 ± 10.3	0.034
At 30 min	170.3 ± 42.1	159.4 ± 31.4	0.058
At 60 min	200.6 ± 70.7	168.0 ± 49.9	0.020
At 90 min	206.3 ± 64.8	168.0 ± 62.0	0.002
At 120 min	179.7 ± 68.5	136.3 ± 49.0	<0.001
At 180 min	153.4 ± 64.2	117.7 ± 59.3	<0.001
Insulin (mI/L)			
At 0 min	14.1 ± 9.4	10.4 ± 5.1	0.011
At 30 min	72.1 ± 36.3	75.1 ± 31.9	0.758
At 120 min	138.4 ± 76.5	102.1 ± 62.8	0.004

HOMA-IR, homeostasis model for insulin resistance; IGI, insulinogenic index.

of the approximately 70 000 subjects without liver damage and kidney damage in our hospital was 4.5 ± 0.3 g/dL, lower limit of normal albumin level was defined as 3.9 g/dL (=mean−2 × standard deviation). On ICG_R15, we divided the patients into two groups based on mean level of 25%. The hypoalbuminemia and elevated ICG_R15 indicates the severity of liver cirrhosis. Thus, our results suggest that severity of liver cirrhosis was the most important factor for predicting T2DM. The reported predictive factors of diabetes mellitus in liver cirrhosis were age, male, BMI, and Child–Pugh score.[9,10,18–21] Quintana *et al.* have reported hypoalbuminemia as risk factor of diabetes mellitus in cirrhotic patients.[22] On the other hand, EL-Serag *et al.* have reported that hepatogeneous diabetes is less frequently associated with risk factors such as age, BMI, and family history of diabetes.[23]

Third, patients with serum albumin level of <3.9 g/dL and ICG $_R$15 of ≥25% revealed high insulin resistance and low insulin secretion compared to patients with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of <25%. This result suggests that insulin resistance and insulin secretion are associated with the onset of diabetes in advanced liver cirrhosis.

The precise mechanism of hepatogeneous diabetes is not precisely known. The possible mechanism is the following: (i) insulin resistance of muscle and adipose tissue; and (ii) impairment of the insulin secretion activity of the beta-cells of the pancreas.[24,25] Our results show the elevated insulin resistance and decrease of insulinogenic index. Thus, our results agreed with the possible mechanism of hepatogeneous diabetes.

The limitation of present study is that our cohort contains Japanese patients only. Thus, the result needs to be confirmed in other ethnic groups. Moreover, in patients with chronic liver disease, HbA1c levels have been seen to be apparently lower than real values due to a shortened half-life of erythrocytes originating from hypersplenism.[26] Thus, we could not evaluate the HbA1c in the present study.

In conclusion, our data suggest that physicians in charge of hepatitis virus related cirrhotic patients with hypoalbuminemia and elevated ICG $_R$15 should pay attention to complication of diabetes.

ACKNOWLEDGMENT

THE PRESENT WORK was supported in part by Grants-in-Aid from of the Department of Health, Labour and Welfare.

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Hepatology Research

Hepatology Research 2014; 44: E267–E272 doi: 10.1111/hepr.12237

Case Report

Sequential occurrence of acute hepatitis B among members of a high school Sumo wrestling club

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A 17-year-old male was admitted to our hospital and diagnosed with acute hepatitis B. Six weeks later, a 15-year-old male was admitted with acute hepatitis B as well. They were Sumo wrestling players in the same club. A detailed survey in the club revealed that a 28-year-old male coach was a hepatitis B surface antigen carrier with high-level viremia. The consistency of hepatitis B virus (HBV) DNA in the infected players was revealed by analyzing the complete HBV genome sequences. Sumo players are more likely to get injured, including cuts and bleeding, compared with players of other sports because of the characteristic wrestling style. Several past reports have suggested that highly viremic HBV carriers have high HBV DNA titers in both their blood and other body fluids such as sweat. In our cases, percutaneous HBV transmission through the bleeding wounds was the most probable infection route. We conclude that a universal HBV immunization program should be introduced urgently in Japan, similar to those implemented in other countries worldwide.

Key words: hepatitis B virus, horizontal transmission, Sumo, universal vaccination

INTRODUCTION

THE HORIZONTAL TRANSMISSION of hepatitis B virus (HBV) occurs in limited situations such as sexual intercourse with HBV positive partners, the transfusion of HBV-contaminated blood, and the re-use of needles and syringes used for the i.v. administration of drugs.[1–3] In addition, there are several reports of horizontal HBV transmission in elementary schools and day-care centers due to bites and scratches or exposure to blood or blood-contaminated fluids among children.[4–7] Although it is rare, HBV horizontal transmission has been reported in various sports as well, including Sumo wrestling and American football, because of contact with open wounds during training.[8,9]

In this paper, we report a sequential occurrence of acute hepatitis B in members of a high school Sumo wrestling club. After a detailed field survey, a 28-year-old male coach was determined to be a hepatitis B surface antigen (HBsAg) carrier with a high-level of viremia. This individual was identified as the source of transmission by analyzing the complete HBV genome sequences.

CASE REPORT

A 17-YEAR-OLD MALE (case 1) was admitted to our hospital with a 1-week history of jaundice and itching. He had no past medical history, except pediatric asthma, and was not taking any medications currently. There were no HBV carriers in his family. He reported no alcohol consumption, recent travel or sexual contact. He was a member of a high school Sumo wrestling club. On examination, the patient was slightly icteric with stable vital signs. Blood test results (Table 1) revealed the following: total bilirubin (T-Bil), 3.9 mg/dL; aspartate aminotransferase (AST), 1152 IU/L; alanine aminotransferase (ALT), 2856 IU/L; HBsAg, 12 229.87 IU/mL; hepatitis B e-antigen (HBeAg), 473.29 S/CO; anti-hepatitis B core (anti-HBc), 4.0 S/CO; immunoglobulin

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Conflict of interest: None.
Financial disclosure: None to declare.
Received 5 August 2013; revision 2 September 2013; accepted 3 September 2013.

Table 1 Laboratory findings at the initial visit

	Case 1	Case 2	Case 3	Reference range
White blood cells (10^3/μL)	5.8	4.0	4.2	3.9–9.8
Red blood cells (10^6/μL)	5.38	5.18	5.11	4.10–5.30
Hemoglobin (g/dL)	15.8	14.7	16.4	13.5–17.6
Platelet (10^3/μL)	293	232	198	131–362
Prothrombin time (%)	100.6	89.7	106.0	72.0–130.0
APTT (s)	38.7	36.6	38.7	24.8–40.4
Total bilirubin (mg/dL)	3.9	3.2	0.9	0.3–1.2
Direct bilirubin (mg/dL)	2.5	2.0	0.1	0.0–0.2
AST (IU/L)	1152	1567	42	13–33
ALT (IU/L)	2856	2526	76	8–42
ALP (IU/L)	729	1167	237	115–359
γ-GT (IU/L)	176	170	46	10–47
Albumin (g/dL)	4.9	4.1	4.9	4.0–5.0
Immunoglobulin G (mg/dL)	1600	1730	1080	870–1700
Immunoglobulin A (mg/dL)	220	297	281	110–410
Immunoglobulin M (mg/dL)	120	107	114	33–190
HBsAg (IU/mL)	12 229.87	12 381.98	62 276.59	<0.05
HBeAg (S/CO)	473.29	553.41	1394.20	<1.00
Anti-HBe (INH%)	0.0	0.0	0.0	<50
Anti-HBc (S/CO)	4.00	7.88	7.34	<1.00
IgM anti-HBc (S/CO)	24.10	19.30	0.78	<1.00
HBV DNA (log copies/mL)	6.1	6.1	>9.1	Non-detectable
HBV genotype	C	C	C	–
Precore mutations	Wild	Wild	Wild	–
Core promoter mutations	Wild	Wild	Wild	–
HCV RNA (log IU/mL)	Non-detectable	Non-detectable	Not tested	Non-detectable
IgM anti-HAV (S/CO)	<0.40	<0.40	Not tested	<0.40
EBV-VCA IgG	0.8	3.3	Not tested	<0.5
EBV-VCA IgM	0.0	0.0	Not tested	<0.5

ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc, anti-hepatitis B core; anti-HBeAg, hepatitis B e-antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; EBV-VCA, Epstein–Barr virus viral capsid antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IgM anti-HAV, immunoglobulin M anti-hepatitis A virus; IgM anti-HBc, immunoglobulin M anti-hepatitis B core; γ-GT, γ-glutamyl transferase.

M anti-hepatitis B core (IgM anti-HBc), 24.1 S/CO; HBV DNA, 6.1 log copies/mL; and HBV genotype, C. On the basis of these results, a diagnosis of acute hepatitis B was confirmed, although the exact route and source of infection could not be identified. The patient recovered naturally and was discharged 12 days after admission. The clinical course was uneventful, and HBsAg clearance was achieved 157 days after admission.

Six weeks after discharge of case 1, a 15-year-old male (case 2) from the same high school Sumo wrestling club was admitted to our hospital with elevated transaminases. He had no past medical history, except atopic dermatitis, and was not taking any medications currently. There were no HBV carriers in his family, except that his father was an inactive HBsAg carrier. He reported no alcohol consumption, recent travel or sexual contact. On examination, the patient was slightly icteric with stable vital signs. Blood test results (Table 1) revealed the following: T-Bil, 3.2 mg/dL; AST, 1567 IU/L; ALT, 2526 IU/L; HBsAg, 12 381.98 IU/mL; HBeAg, 553.41 S/CO; anti-HBc, 7.88 S/CO; IgM anti-HBc, 19.30 S/CO; HBV DNA, 6.1 log copies/mL; and HBV genotype, C. On the basis of these results, this individual was diagnosed with acute hepatitis B as well. However, as in the first case, we could not identify the precise route or source of infection. The patient recovered naturally and was discharged 30 days after admission. The clinical course was uneventful, and HBsAg clearance was achieved 96 days after admission.

Because acute hepatitis B was observed to occur successively in the same high school Sumo wrestling club in a relatively short time period, we suspected the presence

of an infection route and source within the club. After obtaining informed consent from all the club members and coaches, they were tested for HBsAg and hepatitis B surface antibody (anti-HBs) by the school health service. Consequently, a 28-year-old male coach (case 3) was observed to be HBsAg and HBeAg positive with a high level of viremia. There were no HBV carriers in his family. His blood test results (Table 1) revealed the following: T-Bil, 0.9 mg/dL; AST, 42 IU/L; ALT, 76 IU/L; HBsAg, 62 276.59 IU/mL; HBeAg, 1394.20 S/CO; anti-HBc, 7.34 S/CO; IgM anti-HBc, 0.78 S/CO; HBV DNA, more than 9.1 log copies/mL; and HBV genotype, C. To identify the infection source, we performed an analysis of the complete HBV genome sequences in the two patients with acute hepatitis B as well as in the coach suspected to be the source of HBV transmission. Three isolates obtained from the two patients (cases 1 and 2) and the coach (case 3) had the same genomic length of 3215. Between case 1 and case 3, one base (nt1272) had mutated from T to G, with 99.97% (3214/3215) HBV DNA being consistent. Further, between case 2 and case 3, the HBV DNA sequence was 100% (3215/3215) consistent. Using Basic Local Alignment Search Tool (BLAST) analysis, which is a sequence similarity search program to compare a query to a database of sequences, we found that the sample from case 1 was most genetically similar to samples from cases 2 and 3 among other pooled samples. A phylogenetic tree of full-length HBV, obtained using a neighbor-joining method, revealed that the three isolates in this study (cases 1, 2 and 3) were most closely related to each other, and classified into subgenotype C2 (Fig. 1). On the basis of these results, the coach was determined to be the infection source for the successive occurrence of acute hepatitis B in this Sumo wrestling club.

DISCUSSION

ALTHOUGH SEXUAL INTERCOURSE is known as the major route for the horizontal transmission of HBV, several other routes have been reported in the past as well. Iatrogenic routes, including dental or oral surgery,[10] sharing of needles,[11] fingerstick blood-sampling devices,[12] surgical procedures[13] and acupuncture,[14] have been revealed as possible routes of horizontal HBV transmission. On the other hand, non-iatrogenic routes for horizontal HBV transmission include bites and scratches in children's day-care centers or institutions for the mentally retarded,[4–7] household contact,[15–19] tattooing,[20] sharing knives among butchers,[21] and needle pricks or scissor cuts in barbers.[22]

With regard to the field of sports, Kashiwagi *et al.* reported an acute hepatitis B outbreak in a high school Sumo wrestling club in 1982.[8] They confirmed five cases of acute hepatitis B among 10 club members within a 1-year period. Investigations identified an asymptomatic carrier who was judged to be the source of transmission for the hepatitis infection that occurred during the training sessions. Thereafter, in 2000, Tobe *et al.* reported an outbreak of acute hepatitis B in an American university football team.[9] During a period of 19 months, they confirmed five cases of acute hepatitis B among 65 team members and detected a single HBeAg carrier in the same training group. Consequently, they concluded that the carrier was the source of the hepatitis infection that occurred during the training sessions. They performed HBsAg analysis (subtype adr) and suggested that horizontal HBV transmission can occur in sports, probably because of contact with open wounds during training.

We also experienced successive occurrence of acute hepatitis B in a high school Sumo wrestling club similar to that reported by Kashiwagi *et al.*[8] We initiated an investigation in the club after confirming the diagnosis in the second patient. Sumo players wrestle on hard soil in an almost naked style, except for the Sumo belt, which is referred to as "Mawashi" in Japanese. Therefore, they are more likely to be injured and incur cuts and bleeding compared with athletes in other sports. Several recent reports have suggested that HBV carriers may exhibit high HBV DNA titers in other body fluids such as sweat, saliva, tears, nasopharyngeal fluid and urine.[23–27] In our cases, we could not determine whether the intermediate for HBV was blood or other body fluids. However, during their daily training, the players take turns wrestling with one another and continue even when injured or while bleeding from wounds. The nature of this training and our test results suggested that HBV was transmitted through cuts and bleeding wounds sustained during training. We eventually identified the carrier as the source of transmission by analyzing the complete HBV genome sequences in the infected patients. Several cases of horizontal HBV transmission have been reported previously; however, in the field of sports, this is the first report that confirmed the consistency of HBV DNA in the infected patients. Meanwhile, identification of the exact route of HBV transmission was difficult in the three patients in this outbreak. The mean incubation period for acute hepatitis B is 2–3 months after exposure but can range 1–6 months after exposure.[28] This implies that it is possible for one of the two patients with acute hepatitis B to have

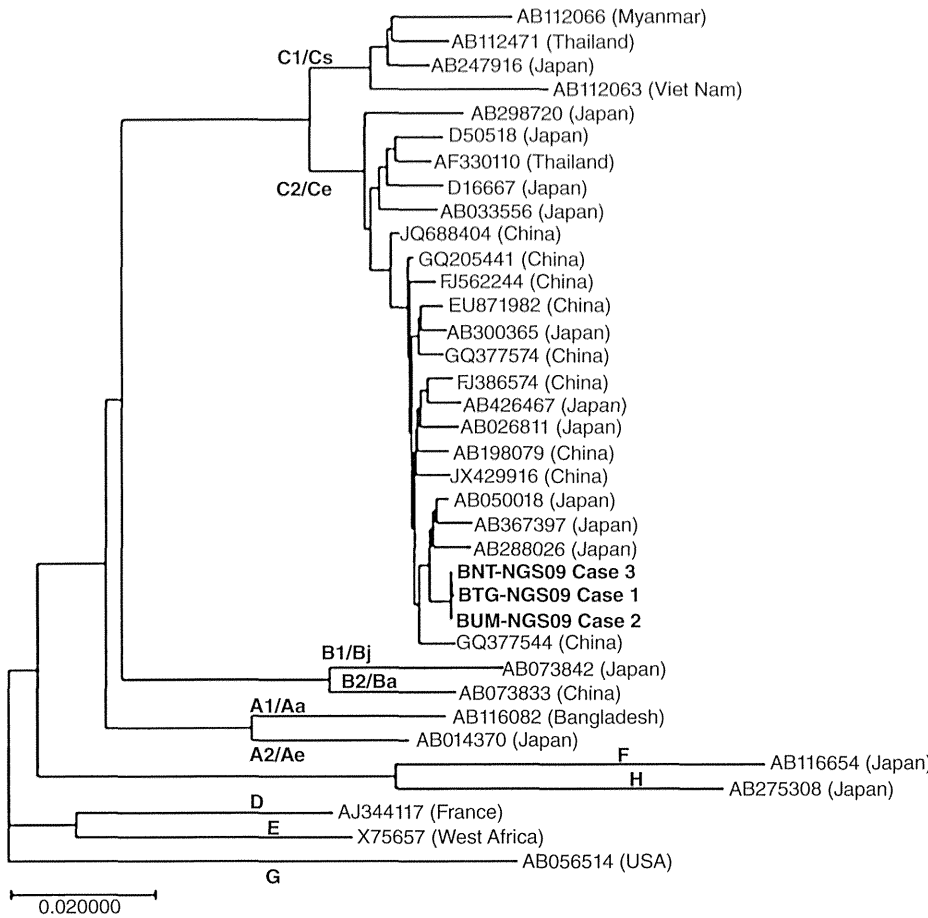

Figure 1 Phylogenetic tree of full-length hepatitis B virus by the neighbor-joining method. Isolates obtained in this study (cases 1, 2 and 3) are shown in bold.

infected the other during the incubation period. In our cases, the coach could have been the origin of transmission and could have infected at least one player, although we could not determine whether or not he infected the other player.

It is remarkable that all three reports of horizontal HBV transmission in the field of sports were from Japan. In 1992, the World Health Organization recommended that all countries should integrate hepatitis B vaccination into their national immunization programs by 1997. By the end of 2009, 177 countries had implemented a universal HBV immunization program for newborns, infants and/or adolescents. However, at the time of drafting of this manuscript (2013), Japan had not introduced this universal HBV immunization program yet. In 1986, Japan initiated a national prevention program comprising selective vaccination for newborns delivered by HBV carrier females. However, this does not aim at preventing horizontal HBV transmission but prevents vertical transmission alone.

Although the number of professional Sumo wrestling players in Japan is very few, the Japanese Ministry of Education revised the guidelines for junior high school education to include compulsory "Budo" (Japanese martial arts) education in 2008. Nowadays, all the students in Japanese junior high schools are taking martial arts classes such as Sumo and Judo. This means that they have a certain risk of exposure to HBV through body fluids or blood during the classes, even though most of them are negative for anti-HBs. In addition, recently

8 million foreign tourists visit Japan and 18 million Japanese nationals travel abroad each year. This has resulted in the rapid development of Japan's internationalization. Consequently, HBV genotype A infections as a sexually-transmitted disease have increased in urban areas of Japan, and then spread to other areas.[29] Thus, this might have increased the risk of horizontal HBV transmission in Japan, particularly in young individuals without HBs antibodies. Therefore, there is an urgent need to prevent horizontal HBV transmission in Japan, and thus the introduction of a universal HBV immunization program is both needed and desirable.

ACKNOWLEDGMENTS

THE AUTHORS ARE grateful to S. Bekki, R. Hamada, M. Fukuda, R. Nakao, T. Hirano and K. Yano for their valuable support in the preparation of this manuscript.

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HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Risk Factors for Long-Term Persistence of Serum Hepatitis B Surface Antigen Following Acute Hepatitis B Virus Infection in Japanese Adults

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The proportion of patients who progress to chronicity following acute hepatitis B (AHB) varies widely worldwide. Moreover, the association between viral persistence after AHB and hepatitis B virus (HBV) genotypes in adults remains unclear. A nationwide multicenter study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in patients with AHB. For comparing factors between AHB patients with viral persistence and those with self-limited infection, 212 AHB patients without human immunodeficiency virus (HIV) coinfection were observed in 38 liver centers until serum hepatitis B surface antigen (HBsAg) disappeared or a minimum of 6 months in cases where HBsAg persisted. The time to disappearance of HBsAg was significantly longer for genotype A patients than that of patients infected with non-A genotypes. When chronicity was defined as the persistence of HBsAg positivity for more than 6 or 12 months, the rate of progression to chronicity was higher in patients with genotype A, although many cases caused by genotype A were prolonged cases of AHB, rather than chronic infection. Multivariate logistic regression analysis revealed only genotype A was independently associated with viral persistence following AHB. A higher peak level of HBV DNA and a lower peak of alanine aminotransferase (ALT) levels were characteristics of AHB caused by genotype A. Treatment with nucleotide analogs (NAs) did not prevent progression to chronic infection following AHB overall. Subanalysis suggested early NA initiation may enhance the viral clearance. *Conclusion*: Genotype A was an independent risk factor for progression to chronic infection following AHB. Our data will be useful in elucidating the association between viral persistence after AHB, host genetic factors, and treatment with NAs in future studies. (HEPATOLOGY 2014;59:89-97)

Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e-antigen; CLIA, chemiluminescent enzyme immunoassay; EIA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M; anti-HBe, antibody to HBeAg; NAs, nucleotide analogs; RPHA, reverse passive hemagglutination.

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Received January 5, 2013; accepted July 10, 2013.