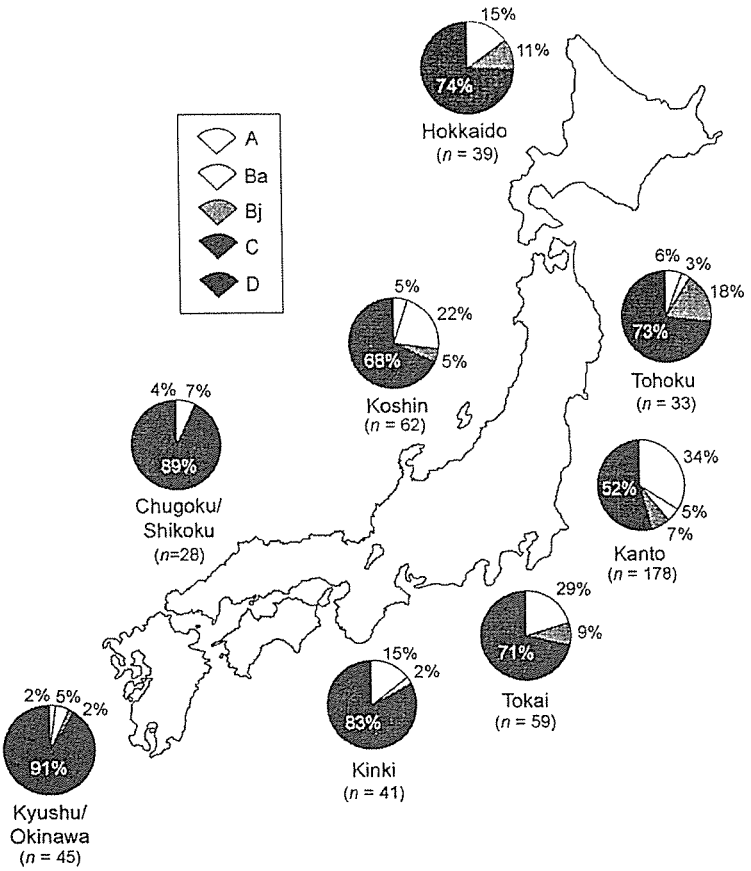

Fig. 1. Geographic distribution of HBV genotypes among 485 patients with acute hepatitis in Japan during 1982–2005.

The core-promoter double mutation (A1762T/G1764A) was more frequent in patients with fulminant than acute self-limited hepatitis infected either with HBV/Bj (70% versus 0%, $p < 0.01$) or HBV/C (50% versus 18%, $p < 0.01$). Also, precore stop-codon mutation (G1896A) was more offen in patients with fulminant than acute self-limited hepatitis who were infected with HBV/Ba (100% versus 5%, $p = 0.01$), HBV/Bj (60% versus 0%, $p < 0.01$) or HBV/C (50% versus 14%, $p < 0.01$).

3.4. Changes in the distribution of HBV genotype with the age and time

Fig. 2 depicts the distribution of HBV genotypes stratified by the age. Prevalence of HBV/A decreased with the age, and was higher in the patients aged <30 years (26%) than in the 40s (14%, $p = 0.03$) or aged ≥50 years (8%, $p < 0.01$). Prevalence of HBV/B increased with the age, in converse, and was lower in the patients aged <30 years (7%) than in the 40s (18%, $p = 0.01$) or aged ≥50 years (17%, $p = 0.02$). No significant differences were observed in the distribution of HBV/Ba or HBV/Bj among all age groups.

Fig. 3 illustrates changes in HBV genotypes through the four time spans covering 24 years. HBV/A accounted for 5% (2/42) in 1982–1990, 5% (4/78) in 1991–1995 and 14% (18/133) in 1996–2000, and thereafter increased to 33% (58/176) in 2001–2005. There was significant difference between 1982–1995 and 1996–2005 ($p < 0.01$). HBV/B accounted for 26% (11/42) in 1982–1990, 17% (13/78) in

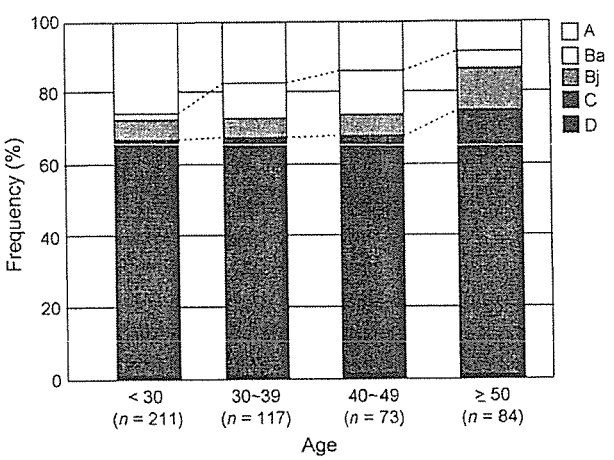

Fig. 2. Age-specific prevalence of HBV genotypes among 485 patients with acute hepatitis.

Table 1
Demographic and clinical differences among patients infected with HBV of distinct genotypes

	Genotypes			
	A ($n=92$)	Ba ($n=26$)	Bj ($n=32$)	C ($n=330$)
Mean age (years)	31.8 ± 10.9[a]	40.7 ± 10.9[b]	41.2 ± 17.0	35.8 ± 13.9
Age <30 years	54 (58%)[c]	3 (12%)[d]	12 (38%)	140 (42%)
Male	85 (92%)[e]	23 (88%)[f]	18 (56%)	210 (64%)
Peak ALT (IU/L)	2051 ± 1009[g]	2536 ± 1104	3371 ± 2342[h]	2650 ± 1747
Peak total				
Bilirubin (mg/dL)	10.3 ± 10.4	8.9 ± 5.8	10.5 ± 8.1	9.3 ± 9.7
HBeAg positive	78 (84%)[i]	14 (54%)	19 (59%)	199 (60%)
Transmission routes				
Sexual	52 (57%)	19 (73%)	11 (34%)	134 (40%)
Medical accident	0	0	3 (9%)	11 (3%)
Drug/tattoo	0	0	0	1 (1%)
Blood transfusion	0	0	1 (4%)	3 (1%)
Unknown	40 (43%)	7 (23%)	17 (53%)	181 (55%)
Metropolitan areas	60 (65%)[i]	8 (31%)	13 (41%)	93 (28%)
Fulminant hepatitis	1 (1%)	2 (8%)	13 (41%)[j]	29 (9%)
Mutations in HBV DNA				
BCP (1762T/1764A)				
Acute self-limited	2/67 (3%)	4/22 (18%)	0/13 (0%)[k]	41/223 (18%)[k]
Fulminant	0/1 (0%)	0/2 (0%)	7/10 (70%)	10/20 (50%)
Precore (1896A)				
Acute self-limited	1/67 (1%)	1/22 (5%)[k]	0/13 (0%)[l]	31/223 (14%)[k]
Fulminant	1/1 (100%)	2/2 (100%)	6/10 (60%)	10/20 (50%)

$p<0.01$, acute vs. fulminant.
[a] $p<0.01$, A vs. Ba. $p=0.01$, A vs. Bj. $p=0.03$, A vs. C.
[b] $p=0.02$, Ba vs. C.
[c] $p<0.01$, A vs. Ba. $p<0.04$, A vs. Bj. $p<0.01$, A vs. C.
[d] $p<0.01$, Ba vs. C. $p<0.04$, A vs. Bj. $p<0.01$, A vs. C.
[e] $p<0.001$, A vs. Bj. $p<0.01$, A vs. C.
[f] $p<0.01$, Ba vs. Bj. $p<0.01$, Ba vs. C.
[g] $p=0.04$, A vs. Ba. $p<0.01$, A vs. B1. $p<0.01$, A vs. C.
[h] $p=0.03$, Bj vs. C.
[i] $p<0.01$, A vs. Ba, Bj or C.
[j] $p<0.01$, Bj vs. A, Ba or C.
[k] $p<0.01$, acute vs. fulminant.
[l] $p=0.01$, acute vs. fulminant.

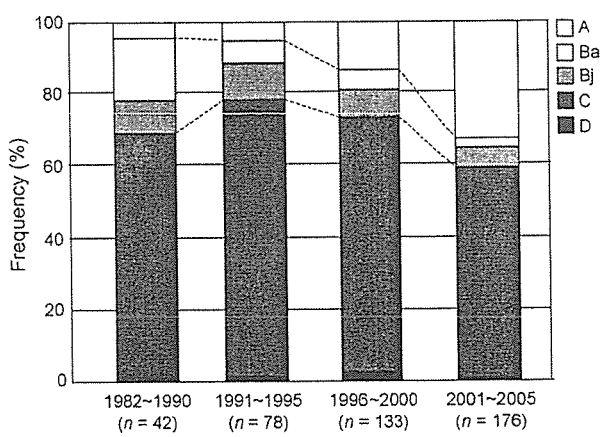

Fig. 3. Chronological changes in the distribution of HBV genotypes among 429 patients with acute hepatitis.

1991–1995 and 14% (18/133) in 1996–2000, and thereafter decreased to 8% (14/176) in 2001–2005; there was significant difference between 1982–1990 and 2001–2005 ($p<0.01$). However, no significant differences were observed in the distribution of HBV/Ba or HBV/Bj among the four time spans.

4. Discussion

As in most Asian countries, the persistent HBV carrier state had been established mainly through perinatal transmission and horizontal infection during the infancy in Japan, until 1986 when combined active and passive immunoprophylaxis was started in the national program. There still are approximately million HBV carriers estimated by the prevalence of HBsAg in the first-time blood donors [28]; they had been infected with HBV before the prophylaxis started. The age-specific prevalence is high in the elderly, and some of them develop chronic liver disease culminating in cirrhosis and hepatocellular carcinoma. By far the majority of genotypes in

patients with persistent HBV infection are HBV/B (approximately 30%) and HBV/C (about 70%) [4,29]. In patients with acute hepatitis in hospitals in the Metropolitan Tokyo, however, HBV/A was detected in 23% with HBV/Ae prevailing [5], in contrast to HBV/B in 14% and HBV/C in 44% [4]. It is not certain if a high prevalence of HBV/A in acute HBV infection in Tokyo is extrapolated to the other areas in Japan.

The present study was performed on 547 patients with acute hepatitis B visiting 25 hospitals in Japan during 24 years from 1982 to 2005. They include the 147 (27%) patients reported by Yotsunangi et al. [7] and the 336 (61%) by Ozasa et al. [22]; the remaining 64 (12%) patients were recruited anew. Yotsuyanagi et al. [7] dealt with a peculiar distribution of HBV/A clustering in and around Metropolitan areas and clinical manifestation dependent on genotypes, while Ozasa et al. [22] focused on the influence of HBV genotypes on clinical outcomes, in terms of fulminant hepatitis and viral persistence, as well as the occurrence of precore stop-codon mutation. The assortment of these cohorts of patients have given us the power to precisely picture the epidemiology of genotypes in acute HBV infection all over Japan and shift thereof during the past quarter of century. In addition, it confirmed the results of previous studies on the precore mutations, and extended them to the core-promoter double mutation that can make differences in fulminant or self-limited outcome of acute HBV infection under the influence of HBV genotypes. In the present multicenter study on patients with acute hepatitis B, HBV/C was the most frequent and HBV/A was the second most common in central areas in Japan harboring big cities (Fig. 1). Along with HBV/A, the other foreign genotypes (Ba and D) accounted for 123 of the 485 (25%) acute HBV infections. Furthermore, foreign origins of acute HBV infections would increase further, if overseas origins of HBV/C (Ce and Cs) are examined in the future.

Clinical relevance of HBV genotypes, in terms of severity of liver disease and response to antiviral treatment, has been evaluated mostly in patients with chronic liver disease [27,30,31]. Due to uneven distributions of HBV genotypes over the world, however, comparison is largely restricted to only two genotypes prevailing in each country. All in all, it would be reasonable to state that patients with HBV/A or HBV/B fare better than those with HBV/D or HBV/C [21,27,30–32]. Influence of HBV genotypes on acute hepatitis B is also reported [33]. In the present study, in which patients with acute hepatitis infected with HBV/A, HBV/Ba, HBV/Bj or HBV/C were compared, there were significant differences in age, gender, peak ALT levels, HBeAg positivity and outcome (Table 1). These results indicate that HBV genotypes influence the clinical profile in not only chronic, but also acute hepatitis B.

Fulminant hepatitis developed rather frequently in 45 of the 485 (9%) patients, although it would be overrepresented in them who visited hospitals due to overt liver disease. Remarkably, chances for patients infected with HBV/Bj to develop fulminant hepatitis were significantly higher than those with HBVA, HBV/Ba or HBV/C (41% versus 1%, 8% or 9%, $p < 0.01$). These results were consistent with those in the previous study [22]. In patients with acute hepatitis B from Chiba in Japan [34], HBV/B was more frequent in those with fulminant than self-limited hepatitis (63% versus 31%, $p = 0.027$); however, some cases of acute exacerbation of persistent HBV infection were included in their study.

The precore stop-codon mutation (G1896A) was detected significantly more frequently in patients with fulminant than acute self-limited hepatitis, in confirmation of our previous report [22]. Furthermore, the BCP double mutation (A1762T/G1764A) was detected more often in fluminant than acute self-limited hepatitis. In infection with HBV/Bj, in particular, the frequency of BCP mutation was much higher in the patients with fulminant (70%) than that reported in those with chronic hepatitis (16%) [27]. Precore and BCP mutations are very frequent in patients with fulminant hepatitis in Asia [35–37] and the Middle East [38], but rare in Western countries [39–41]. These differences may be explained by distinct geographical distributions of HBV genotypes.

An extremely wide range (0–12%) has been reported in the rate of persistence after acute HBV infection in the adulthood [42–49]. Why such a big difference arises needs to be clarified, because the HBV genotype is implicated in a high chronicity rate in European countries where HBV/A is predominant. In accordance with this view, HBV/A was more frequent than HBV/D in 32 patients with chronic active hepatitis (80% versus 11%), while the reverse was the case in acute hepatitis (10% versus 80%) in Switzerland [33]. In Japan, also, infection with HBV/A tends to prolong longer than that with HBV/B or HBV/C in the adulthood (23% [3/13] versus 13% [1/8] or 12% [3/25]) [4]. In the present series on 464 patients with acute hepatitis B in Japan, for whom genotyping was feasible, HBV infection persisted in only 5 (1%) representing 3 of the 92 (3%) with HBV/A, 1 of the 58 (2%) with HBV/B and 1 of the 330 (0.3%) with HBV/C. Hence the infection with HBV/A would persist longer than those with the other genotypes.

The present study highlighted the recent rapid increase in cases of acute hepatitis B in Japan, which gained about five-fold from 4.7 per year before 1990 to 33.1 after 2000 in participant hospitals. Primary HBV infection in the adulthood can occur by homo/heterosexual contacts, intravenous drug use, medical accidents and blood transfusion [50–54]. Acute HBV infection in the 485 patients studied was transmitted by sexual contacts in 216 (45%), medical accidents in 14 (3% (none since 2001)) and blood transfusion in 4 (1% (none since 2001)). The route is unknown for the remaining 245 (51%) patients; some of them might have been infected by undeclared sexual contacts and/or intravenous drug use, although recreational drugs are still uncommon in Japan. It comes as a surprise that the overall proportion of foreign genotypes (A, Ba and D) in patients with acute hepatitis B was high at 25%. Of these foreign genotypes, Asian genotypes such as HBV/Ba have decreased gradually, while HBV/A has increased in converse (Fig. 3). Furthermore,

some of "domestic" HBV/C infections that accounted for the majority (68%) may well have been imported by sexual workers from foreign countries where this genotype is prevalent. Combined, a substantial part of acute HBV infection in Japan does seem to have been imported.

In conclusion, the distribution of HBV genotypes in patients with acute hepatitis B is different geographically, and has changed with time in Japan. Recently, acute HBV infection is increasing among the Japanese adults, although it rarely becomes chronic. A significant part of it is transmitted sexually with foreign HBV genotypes. Furthermore, a fulminant outcome is frequent after infection with HBV/Bj having precore and/or BCP mutations. These facts should be made open to the public for making them aware of the risk and taking measures to prevent it.

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Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection

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Abstract

Background: Prolonged lamivudine therapy has two major problems: breakthrough hepatitis during treatment and relapse of aminotransferase (ALT) after cessation of the therapy. The aim of this study was to examine factors that could predict ALT flare after stopping lamivudine therapy.
Methods: We analyzed 22 Japanese patients with chronic hepatitis B infection, in whom lamivudine therapy was stopped after HBV DNA level had been gone undetectable (<3.7 LGE/ml) during at least six consecutive months. The post-treatment followed up was carried for 28 months in median (range 9–41). HBV core-related antigen (HBcrAg) assay was assessed using newly developed assay.
Results: After cessation of lamivudine therapy, 11 patients (50%) had relapsed (reactivation of serum ALT >80 IU/l, relapsers) and remaining 11 (50%) did not relapse (non-relapsers). In the univariate comparison of relapsers versus non-relapsers, HBcrAg level at lamivudine cessation point (4.5 ± 1.0 versus 3.4 ± 0.9; $p = 0.0145$) has been shown as a significant predictive factor for non-relapse. All patients with HBcrAg <3.0 log U/ml at the cessation point had no ALT flares. Multivariate analysis on effects of 10 factors (age, sex, cirrhosis, pretreatment ALT level, HBV DNA level, HBcrAg level, mean months till undetectable HBV DNA, duration of undetectable HBV DNA and HBcrAg level at lamivudine cessation point), indicated that HBcrAg level at lamivudine cessation point <3.4 log U/ml was the only independent predictive factor for absence of the post-treatment relapse.
Conclusions: HBcrAg level at lamivudine cessation point might be useful as a prognostic predictor of response to lamivudine therapy cessation. The measurement of HBcrAg is a useful additional test for monitoring chronic HBV infection.

Keywords: Hepatitis B virus; Hepatitis B virus core-related antigen; Lamivudine; Chronic hepatitis B virus infection

1. Introduction

Chronic hepatitis B virus (HBV) infection remains to be one of the major global health problems, affecting an estimate of 400 million people worldwide [1]. In a significant proportion of cases, chorinic infection progress to cirrhosis and liver failure as well as hepatocellular carcinoma (HCC) [2]. Lamivudine monotherapy is considered to be a therapeutic option for patients with chronic hepatitis B, irrespective of hepatitis B e antigen (HBeAg) status [3]. Viral resistance or viral breakthrough frequently associated in with prolongation of lamivudine therapy and caused by drug-resistant HBV mutants [4–7]. The breakthrough hepatitis may develop acute

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hepatitis flares, hepatic decompensation, and fatal hepatic failure [8–10]. Furthermore, the occurrence of lamivudine resistance increases the risk of HCC in antibody to HBeAg (anti-HBe) positive cirrhosis [11]. Hence, the Asia–Pacific consensus on the prevention and management of chronic hepatitis B and C has recommended that full HBeAg seroconversion, defined as undetectable HBeAg and HBV DNA with reappearance of anti-HBe, may be considered a suitable end point for lamivudine therapy. It is also recommended that lamivudine therapy should be maintained for 4–6 months after achieving HBeAg seroconversion to decrease the chance of posttreatment relapse [12].

Our recent study showed that highly sensitive HBV real-time detection direct test (<0.7 log IU/ml) can predict non-relapse after cessation of lamivudine monotherapy for chronic HBV infection [13], but the HBV real-time detection direct test is technically complicated and costly. On the other hand, an enzyme immunoassay (EIA) is a relatively simple method and provides a low cost and quantitative analysis with high reproducibility might have advantages over nucleic acid amplification assay if implied as an alternative. Recently, a chemiluminescence enzyme immunoassay (CLEIA) was developed for detection of hepatitis B virus core-related antigen (HBcrAg), which have positive correlation with HBV DNA levels in serum [14,15] and hepatocytes (submitted for publication). Furthermore, HBcrAg was shown as a clinically significant marker in monitoring the antiviral effect of lamivudine therapy [15,16]. Present study evaluated the usefulness of monitoring HBcrAg as a prognostic predictor of lamivudine therapy cessation in HBV-infected patients.

2. Patients, materials, and methods

2.1. Patients

A total of 22 patients with chronic hepatitis B, who were receiving lamivudine therapy during 1996 and 2005 at Nagoya City University Hospital (Nagoya) were enrolled in this study. Cirrhosis was determined mainly by ultrasonography (coarse liver architecture, nodular liver surface, and blunt liver edges) and evidence of hypersplenism (splenomegaly on ultrasonography), a platelet count <100,000 mm^{-3}, or a combination thereof. Written informed consent was obtained from each patient. All were administered 100 mg of lamivudine per day for ≥6 months (median, 12 months; range, 6–43 months). The lamivudine therapy was stopped for each of the patients who had HBV DNA level maintained to be undetectable (<3.7 log genome equivalents [LGE]/ml), as measured by transcription-mediated amplification–hybridization protection assay (TMA–HPA; Chugai Diagnosis science) during >6 consecutive months of the follow up, whereas serum alanine aminotransferase (ALT) level did not exceed 40 IU/l (i.e., the upper limit of normal) during same period. In the HBeAg-positive patients, in addition to undetectable HBV DNA and normal ALT level, HBeAg-seronegative maintained for ≥6 months was used as another criterion for stopping lamivudine therapy.

2.2. Methods

Serum HBsAg, HBeAg, and anti-HBe were measured by commercially available chemiluminescent enzyme immunoassay kit (Fujirebio Inc., Tokyo, Japan). The levels of HBV DNA in serum were determined using the TMA–HPA assay (Fujirebio Inc., Tokyo, Japan), with detection range is 3.7–8.7 LGE/ml and ALT were tested every months. HBcrAg was measured in serum using the CLEIA described previously [14]. Briefly, 150 μl of serum was incubated with 150 μl pretreatment solution containing 15% sodium dodecylsulfate at 60 °C for 30 min. After incubation at 60 ± 4 °C for 30 min, 150 μl pretreated specimen was added to the ferrite micro-particle (coated with monoclonal antibodies (HB44, HB61, and HB114) against denatured HBc and HBe antigens) solution in assay tube. After washing, two other alkaline phosphatase-labelled monoclonal antibodies against denatured HBcAg and HBeAg were added as secondary antibodies. Two hundred microliters substrate (AMPPD: (3-(2′-spiroadamantan)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane disodium salt) (Applied Biosystems, Bedford, MA, USA) solution was added and the assay tube was incubated for 5 min at 37 °C. HBcrAg assay with The relative chemiluminescence intensity was measured with chemiluminescent enzyme immunoassay (CL-EIA) system for fully automated Lumipulse f CL-EIA analyzer (Fujirebio Inc., Tokyo, Japan), and the HBcrAg concentration was estimated by comparison to a standard curve generated using recombinant HBeAg. In the present study, the cutoff value was tentatively set at 3.0 log U/ml. Sera containing over 7.0 log U/ml of HBcrAg were diluted 10- or 100-fold in normal human serum and re-tested to obtain the end titer.

The end point of follow up was relapse after cessation of lamivudine therapy. Relapse was defined as reappearance of serum HBV DNA (≥3.7 LGE/ml, as measured using the TMA–HPA assay) plus a reactivation in the serum ALT to >80 U/l.

2.3. Statistical evaluation

Data are expressed as mean ± S.D. or median (range). The primary focus of this analysis was to compare patients who experienced relapse with those who did not. The Mann–Whitney *U*-test was utilized to analyze quantitative data, the χ^2 test was used for qualitative data. The effect of age, sex, prevalence of cirrhosis, presence of HBeAg, pretreatment ALT level, pretreatment HBV DNA level, pretreatment HBcrAg level, mean months till undetectable HBV DNA, duration of HBV DNA level of <3.7 LGE/ml, duration of lamivudine therapy, and HBcrAg level at cessation point were assessed by logistic regression analysis. *p*-Value of less than 0.05 were considered to be statistically significant.

Table 1
Characteristics of nominated 22 hepatitis B virus (HBV)-infected patients before lamivudine therapy

Characteristic	Value
Age, mean (years) ± S.D.	45.0 ± 9.3
Male sex, no. (%) of patients	12 (55)
HBeAg positive, no. (%) of patients	6 (27)
Genotype/subgenotype (Bj:Ce)	2:20
Cirrhosis, no. (%) of patients	4 (18)
Pretreatment ALT level, median U/l (range)	118 (47–554)
Pretreatment HBV DNA level, median LGE/ml (range)	6.9 (4.1–8.6)
Pretreatment HBcrAg level, median log U/ml (range)	5.8 (4.2–8.6)
Duration of lamivudine therapy, median months (range)	12 (6–43)
Posttreatment follow-up duration, median months (range)	28 (9–41)

Statistical analyses were performed using STATA Software (Stata Corporation, Texas, USA) version 7.0.

3. Results

Table 1 shows the pretreatment demographic and clinical characteristics of the 22 HBV-infected patients who received lamivudine therapy and stopped it. Before the lamivudine administration, serum HBV DNA was detectable in all patients, and HBeAg was positive in six (27%) of the them. HBV genotype B was determined in two patients and remaining 20 had genotype C-infection. All of the six HBeAg-positive patients stopped lamivudine therapy when their HBeAg became negative and their ALT levels were normalized and their HBV DNA levels were undetected by TMA–HPA (<3.7 LGE/ml) for more than 6 months. For the remaining 16 HBeAg-negative patients lamivudine administration was stopped after ALT levels were normalized and their HBV DNA levels were undetected by TMA–HPA for more than 6 months. After cessation of lamivudine therapy, 11 patients (50%) experienced relapse (relapsers) and 11 patients (50%) did not (non-relapsers). All patients were then clinically observed after cessation of lamivudine monotherapy (median duration of posttreatment monitoring, 28 months; range, 9–41 months) (Table 2).

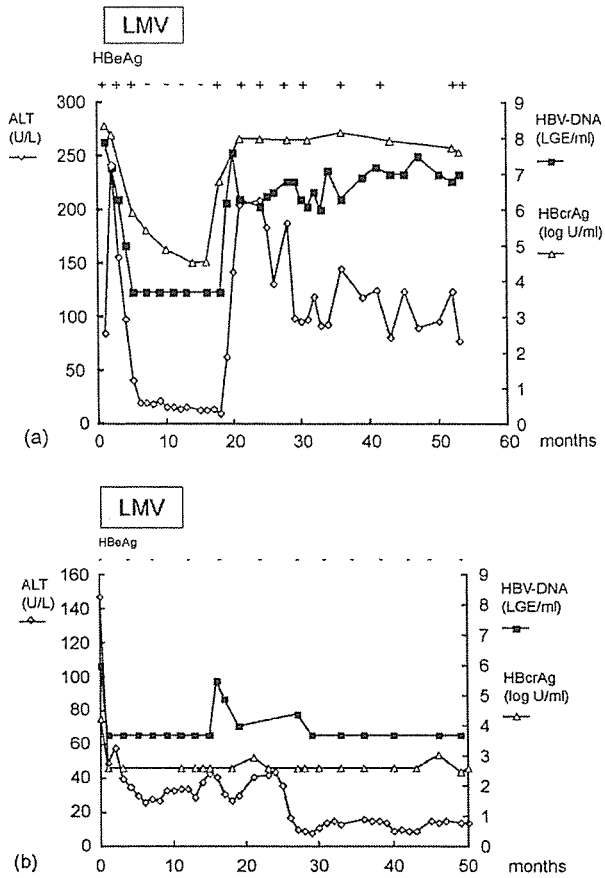

Fig. 1. Featured changes in HBcrAg, HBV DNA and ALT levels observed during clinical courses in (a) patient (no. 1) with ALT relapse and (b) patient (no. 2) without ALT relapse after the cessation of lamivudine therapy.

Fig. 1 shows typical cases with and without relapse after cessation of lamivudine therapy. Case no. 1 (Fig. 1a): a 46-year-old male with chronic hepatitis B (genotype C). Before receiving lamivudine, his laboratory data were as

Table 2
Characteristics patients with and without relapse after lamivudine therapy

Characteristic	Relapsers ($n = 11$)	Non-relapsers ($n = 11$)	p
Age, mean (years) ± S.D.	44.8 ± 9.3	45.2 ± 9.8	2.82
Male sex, no. (%) of patients	4 (36)	8 (72)	0.08
HBeAg positive, no. (%) of patients	3 (27)	3 (27)	>0.999
Genotype Bj, no. (%) of patients	0 (0)	2 (18)	0.48
Cirrhosis	3 (27)	1 (9)	0.59
Pretreatment ALT level, mean (U/l) ± S.D.	118 ± 78	223 ± 159	0.11
Pretreatment HBV DNA, mean (LGE/ml) ± S.D.	6.6 ± 1.1	6.5 ± 1.3	0.87
Pretreatment HBcrAg level, mean (log IU/ml) ± S.D.	6.0 ± 1.1	5.5 ± 1.3	0.26
Mean months till undetectable HBV DNA (months)	2.3 + 1.6	2.2 + 1.7	0.90
Duration of HBV DNA level of <3.7 mean LGE/ml (months)	13.2 ± 4.2	13.5 ± 11.0	0.15
HBcrAg level at lamivudine cessation point, mean (log U/ml) ± S.D.	4.5 ± 1.0	3.4 ± 0.9	0.0145
Duration of lamivudine therapy, median months (range)	13 (11–28)	11 (6–46)	0.18
Posttreatment follow-up duration, median months (range)	29 (9–39)	23 (9–36)	0.32

follows: HBeAg positive; anti-HBe negative; ALT 242 IU/l; HBV DNA 7.9 LGE/ml; HBcrAg 8.36 log U/ml. The level of ALT decreased and was normalized at 4 months after treatment initiated. The level of HBV DNA decreased rapidly to undetectable at 4 months after treatment initiated, while HBcrAg levels decreased more slowly. At 7 months after treatment initiated, HBeAg became negative. Lamivudine therapy was stopped at 17 months after treatment initiated (undetectable HBV DNA for 13 months). At the point, HBcrAg level was 4.54 log U/ml. One month after treatment stopped, HBeAg was reemerged and HBV DNA was 6.2 LGE/ml. Two months after treatment stopped, the patient experienced ALT relapse (200 IU/l). Case no. 2 (Fig. 1b): a 59-year-old male with chronic hepatitis B (subgenotype Bj). Before receiving lamivudine, his laboratory data were as follows: HBeAg negative; anti-HBe positive; ALT 147 IU/l; HBV DNA 6.0 LGE/ml; HBcrAg 4.25 log U/l. The level of ALT decreased and was normalized at 1 month after treatment initiated. The level of HBV DNA decreased rapidly and became undetectable at 1 month after treatment initiated. HBcrAg level also decreased to less than 3.0 log U/ml next month after treatment start. Lamivudine therapy was stopped at 14th months after treatment initiation. At the point, HBcrAg level was less than 3.0 log U/ml. Next month after treatment stopped, HBV DNA became detectable (5.5 LGE/ml) but has decreased; at 6 months after treatment stopped, the level of the HBcrAg remained less than 3.0 log U/ml. ALT was normal during 36 months after treatment stopped. Interestingly, two patients with subgenotype Bj in this study had no ALT relapse after the cessation of lamivudine therapy, probably due to lower pre-treatment HBV DNA and HBcrAg levels as well as persistently undetectable post-treatment HBcrAg levels (<3.0 log U/ml).

In a univariate comparison of patients who experienced relapse (relapsers) with those who did not (non-relapsers), a predictive factor for the non-relapse after cessation of lamivudine was HBcrAg level at the end of treatment (4.5 ± 1.0 versus 3.4 ± 0.9; $p = 0.0145$). The association with age, sex, presence of HBeAg before treatment, presence of cirrhosis, pretreatment ALT level, pretreatment HBV DNA level, pretreatment HBcrAg level, mean months till undetectable HBV DNA, duration of undetectable HBV DNA level (<3.7 LGE/ml), duration of lamivudine therapy was not significant. Interestingly, all six patient whose HBcrAg level <3 log U/ml at the end of treatment did not experienced relapse. Further multivariate analysis involving the 10 above factors effectors indicated that HBcrAg level of <3.4 log U/ml (OR, 103; 95% CI, 1.3–8242; $p = 0.042$), which was a mean value of non-relapsers, was the only independent predictive factor for absence of posttreatment relapse (Table 3). Six of seven patients whose HBcrAg level <3.4 log U/ml at the end of treatment did not experienced relapse [positive predictive value (PPV), 86%]. Ten of 15 patients whose HBcrAg level ≥3.4 log U/ml at the end of treatment experienced relapse [negative predictive value (NPV), 75%], which was higher than that (58%) when the HBcrAg level ≥3.0 log U/ml was applied as cut-off value.

Table 3
Predictive factors for non-relapse in multivariate analysis

Factor	OR (95% CI)[a]	p
Age (years)	0.16 (0.90–1.10)	0.157
Male sex (%)	0.15 (0.001–17.2)	0.429
Cirrhosis (%)	19.4 (0.08–4932)	0.294
Pretreatment ALT level of > 80U/l (%)	1.10 (0.02–63.7)	0.964
Pretreatment HBV DNA (LGE/ml)	0.81 (0.05–14.6)	0.886
Pretreatment HBcrAg level (log U/ml)	1.17 (0.24–5.64)	0.846
Mean months till undetectable HBV DNA (months)	1.32 (0.16–11.1)	0.800
Duration of HBV DNA level of <3.7mean LGE/ml (months)	1.83 (0.15–22.4)	0.636
HBcrAg level of <3.4 log U/ml at lamivudine cessation point	103 (1.3–8242)	0.042
Duration of lamivudine therapy, mean (months) ± S.D.	0.53 (0.42–6.5)	0.616

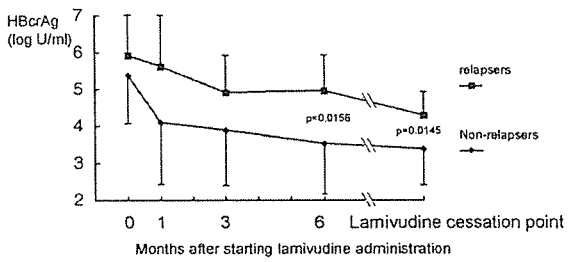

Fig. 2. Linear reduction of the HBcrAg levels is represented in mean value; comparison between relapsers and non-relapsers. At 6 months after treatment initiation and at the cessation point of lamivudine therapy, HBcrAg levels in the patients who experienced relapse were significantly higher than level of HBcrAg in the patients who did not.

Fig. 2 shows comparison of changes in the median levels of HBcrAg during lamivudine administration between relapsers and non-relapsers. The initial median level of HBcrAg in the relapsers was relatively higher than that in the non-relapsers. The levels of HBcrAg in the relapsers were significantly higher than that in the non-relapsers at 6 months after treatment initiation and at cessation points (5.0 ± 1.0 versus 3.5 ± 1.4; $p = 0.0156$ and 4.5 ± 1.0 versus 3.4 ± 0.9; $p = 0.0145$). Interestingly, log-reduction of HBcrAg levels at 6 months after lamivudine administration tended to be greater in non-relapsers than in relapsers (1.7 ± 0.9 versus 1.2 ± 0.8), but no significant. Similarly, log-reduction of HBcrAg at the cessation point also tented to be greater in non-relapsers than in relapsers (2.0 ± 0.9 versus 1.6 ± 1.1), but no significant.

4. Discussion

In this study, we showed that the HBcrAg level <3.4 log U/ml at the point of cessation of lamivudine monotherapy was the only independent predictive factor for absence of post-treatment relapse and better predictive value (PPV 86% NPV 75%) The CLEIA for the HBcrAg is eas-

ier and less costly approach in comparison to PCR-based or signal amplification assays and at the same time is more sensitive than common EIA. Furthermore, it is associated with lower risk of contamination and errors than the nucleic acid amplification assays. The chemiluminescence-based detection system is performed with an automatic microplate reader, takes only 1 h, and does not require a specially trained operator to perform the assay. It also allows quantitative analysis with high reproducibility [14].

Serum HBcrAg levels reflects the viral load in the natural course because these levels positively correlate with those of HBV DNA [14,15]. On the other hand, the character of HBcrAg is somewhat different from that of HBV DNA in patients undergoing anti-viral therapies such as lamivudine. That is, HBcrAg levels decrease significantly more slowly than those of HBV DNA after initiation of lamivudine administration [16]. One of the reasons is that HBcrAg concentration would correlate well with intrahepatic parameters such as Knobell necroinflammation and fibrosis scores, percentage of cells immunostained positive for cytoplasmic and nuclear HBcAg, and intrahepatic total HBV DNA and covalently closed circular DNA (cccDNA level) (submitted for publication).

Lamivudine is a potent nucleoside analogue which inhibits HBV reverse transcriptase, reducing production of HBV DNA-containing virions [17]. As the transcription or translation of viral mRNA is not inhibited by nucleoside analogue such as lamivudine, the production and secretion of HBeAg is not directly inhibited and may persist for a period of time. Moreover, it has been demonstrated that a 22 kDa truncated precore/core protein, which is also detected by the CLEIA assay for HBcrAg, is found in "empty" HBV DNA-negative Dane particles [18]. The production of these particles and HBeAg is not dependent on the formation of HBV DNA, but it reflects the level of transcription and translation of the HBV core/precore gene in the liver. Thus, it is reasonable to monitor HBcrAg level during lamivudine therapy, because the levels of HBcrAg may reflect an altered HBV replication status within the hepatocyte; lower HBcrAg levels in serum corresponding to lower cccDNA levels in hepatocyte could predict non-relapse after the cessation of lamivudine therapy.

In summary, the measurement of HBcrAg is a useful additional test for monitoring chronic HBV infection, allowing continuous monitoring especially in lamivudine-treated patients whose HBV DNA become undetectable by PCR.

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LETTER

Interferon-β plus ribavirin for patients with hepatitis C virus genotype 1: a randomised pilot trial

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Interferon-β plus ribavirin for patients with hepatitis C virus genotype 1: a randomised pilot trial

The rate of sustained eradication of hepatitis C virus (HCV) in response to a combination of interferon-α and ribavirin remains unsatisfactory in patients with genotype 1 infection.[1] No effective alternative treatment is currently available for non-responders. Interferon-β is also a type I interferon commonly used to treat chronic HCV infection in Japan. A previous study showed that a 24 week course of therapy with interferon-β plus ribavirin resulted in sustained loss of HCV in three of nine patients with chronic hepatitis C.[2] However, the efficacy and safety of interferon-β combined with ribavirin has yet to be fully evaluated.

We report the results of a randomised pilot trial comparing interferon-β plus ribavirin with interferon-α plus ribavirin in patients with HCV genotype 1 who poorly responded to interferon-α plus ribavirin. A total of 28 patients with HCV genotype 1 were given 6 MU of recombinant interferon-α2b (Schering-Plough, Kenilworth, New Jersey, USA) by intramuscular injection daily for four weeks. Twenty seven patients (16 men and 11 women; mean age 47 (±8) years) in whom HCV RNA was detected in serum on polymerase chain reaction at week 2 were included in this study and randomly assigned to receive one of two regimens from week 5. Fifteen patients continued to receive 6 MU interferon-α2b intramuscularly, given daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-α group). The other 12 patients were assigned to 6 MU natural interferon-β (Toray Industries Inc., Tokyo, Japan), given by intravenous injection daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-β group). Ribavirin (Schering-Plough) was concurrently administered at a daily dose of 600 mg to patients who weighed 60 kg or less and 800 mg to those who weighed more than 60 kg. At the time of this study, a 24 week course of interferon-α plus ribavirin was commonly used in Japan. The data were analysed according to intention to treat.

Baseline characteristics of the patients in the treatment groups were similar. At week 4 of therapy, when treatment was randomly assigned, the proportion of patients without detectable HCV RNA in serum did not differ between the interferon-α group and interferon-β groups (table 1). The proportion of patients without HCV RNA in serum was higher in the interferon-β group than in the interferon-α group at week 12, but did not differ between the groups at the end of treatment (week 24). However, 24 weeks later (week 48), the proportion of patients with a sustained virological response was significantly higher in the interferon-β group than in the interferon-α group. During treatment, neutralising antibodies to interferon were detected in two patients in the interferon-α group and in no patients in the interferon-β group. Leucocyte, neutrophil, and platelet counts and haemoglobin concentrations were similar in two groups. Therapy was discontinued because of serious adverse events (including depression) in three patients in the interferon-α group; all 12 patients in the interferon-β group completed 24 weeks of treatment. The dose of ribavirin was reduced because of anaemia in eight patients in the interferon-α group and in four in the interferon-β group.

We enrolled patients who did not have a favourable early response to treatment with interferon-α and ribavirin. Antibodies to interferon, which sometimes develop in patients given recombinant interferon-α, can cause resistance to therapy. Both interferon-α and -β bind to a common type I interferon receptor but utilise different regions of the receptor subunits for specific signalling pathways,[3] potentially leading to distinct biological responses. An oligonucleotide array study has shown that some interferon stimulated genes are preferentially induced by interferon-β, but not by interferon-α.[4] We thus believe that interferon-β might be beneficial for some patients who are resistant to interferon-α. A large randomised trial of peginterferon-α plus ribavirin versus interferon-β plus ribavirin for 48 weeks is being conducted in patients with HCV genotype 1 who do not have a virological response[5] to 12 weeks of treatment with peginterferon-α and ribavirin.

In summary, a combination of interferon-β and ribavirin produced a significantly better sustained virological response than a combination of interferon-α and ribavirin in patients with HCV genotype 1 who were resistant to interferon-α plus ribavirin. Although the overall safety profiles of the two regimens were similar, the rates of treatment discontinuation and of reduction in the dose of ribavirin were lower in patients receiving interferon-β and ribavirin than in those receiving interferon-α and ribavirin.

Table 1 Proportions of patients without detectable hepatitis C virus RNA in serum

	Interferon-α group (n = 15)	Interferon-β group (n = 12)	p Value (χ^2 test)
Week 4	4 (27%)	3 (25%)	0.92
Week 12	7 (47%)	10 (83%)	0.049
Week 24 (end of therapy)	10 (67%)	9 (75%)	0.64
Week 48	0 (0%)	3 (25%)	0.040

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REVIEW

Hepatitis B Virus Genotypes and Response to Antiviral Therapy

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SUMMARY

Hepatitis B virus (HBV) infection is an important health problem worldwide. The virus has been classified according to 8 genotypes (A–H) based on sequence divergence. Most genotypes have specific geographic distributions; genotypes A and D are prevalent in Western Europe and North America, and genotypes B and C are prevalent in East Asia and Oceania. Currently accepted treatment for chronic hepatitis B includes interferon alpha, or the nucleoside/nucleotide analogues lamivudine and adefovir. The impact of HBV genotypes on response to antiviral therapy has been studied. HBV genotypes D and C are associated with a lower rate of favorable response to interferon alpha therapy than genotypes A and B, respectively. A study in Germany suggested that the rate of resistance to lamivudine was higher in patients with HBV genotype A infection than in patients with genotype D infection. No difference in the risk of lamivudine resistance is found between patients with genotype B and patients with genotype C. In patients with genotype C infection, however, virological response is worse during lamivudine therapy, and is also less durable after the discontinuation of therapy than in patients with genotype B infection. Determining the genotype could be helpful for predicting the outcome of antiviral therapy in patients with chronic hepatitis B. (Clin. Lab. 2006;52:43-47)

KEY WORDS

hepatitis B, genotype, adefovir, antiviral therapy, interferon, lamivudine

INTRODUCTION

Hepatitis B virus (HBV) affects more than 350 million people worldwide. It is the major causative agent for chronic hepatitis, cirrhosis, and hepatocellular carcinoma, accounting for 1 million deaths annually [1,2].

Currently available antiviral therapy for chronic hepatitis B includes the immunomodulator interferon alpha, and the nucleoside/nucleotide analogues lamivudine and adefovir dipivoxil [3,4]. In addition, entecavir has recently been licensed in the United States. A number of other nucleotide analogues, such as tenofovir, emtricitabine, telbivudine and clevudine, are now at the stage of clinical trials. Interferon alpha is expensive, and is often poorly tolerated. Long-term treatment with nucleoside/nucleotide analogues may induce the emergence of drug-resistant variants with mutations in the reverse transcriptase (rt) domain of the HBV polymerase gene. For instance, mutations from methionine to isoleucine, valine or serine at amino acid rt204 in the tyrosine-methionine-aspartate-aspartate (YMDD) motif are associated with resistance to some nucleoside/nucleotide analogues including lamivudine [5–8].

HBV was traditionally classified into four subtypes (*adr*, *adw*, *ayr*, and *ayw*) based on antigenic determinants of hepatitis B surface antigen [9]. According to the Paris workshop on hepatitis B surface antigen subtypes, these subtypes were further divided into eight types (*adr*, *ayr*, *ayw1*, *ayw2*, *ayw3*, *ayw4*, *adw2*, and *adw4*) [10]. However, the clinical significance of HBV subtypes has not been established. With recent advances in molecular biology techniques, HBV has been classified according to 8 genotypes (A–H) based on sequence divergence in the entire genome exceeding 8% [11–14]. Most genotypes have specific geographic distributions. Genotype A is prevalent in Northwest Europe (including Germany), North America, and Central Africa. Genotypes B and C are common in Southeast Asia, China, Japan, and Oceania. Genotype B is further divided into two subgroups: "B*j*" (corresponds to "B1" of the classi-

fication of Norder et al. [15]) which is exclusive to Japan, and "B*a*" which is common in other countries in Asia [16]. Genotype D prevails in South Europe, the Middle East, and India, although this type has an almost worldwide distribution.

The contribution of HBV genotypes to therapeutic outcome has recently attracted the interest of researchers and clinicians. The clinical outcome of chronic HBV infection has been compared between genotypes A and D in Western countries, and between genotypes B and C in Asia. In general, genotypes D and C are associated with more severe liver disease than genotypes A and B, respectively [17,18]. The impact of HBV genotypes on the response to antiviral therapy has also been studied. In this article, we review the role of HBV genotypes with particular reference to the implications for therapy.

HBV GENOTYPES AND RESPONSE TO INTERFERON ALPHA

Zhang et al. [19] studied HBV genotypes in 35 hepatitis B e antigen (HBeAg)-negative chronic hepatitis B patients in France who had been treated with interferon alpha for a mean duration of 16 months (range, 3–40 months). The response to interferon, defined as sustained normalization of serum alanine aminotransferase (ALT) levels and significant decrease of viremia levels, was found in 7 (70%) of 10 patients with genotype A infection, compared with 10 (40%) of 25 patients with genotype D or E infection ($p = 0.001$). The rate of response to interferon was independent of both initial serum viral DNA levels and interferon doses.

In Germany, Erhardt et al. [20] determined the efficacy of interferon alpha in 165 consecutive patients with chronic replicative hepatitis B with different genotypes. Among them, 119 patients (72%) had HBeAg-positive and 46 (28%) had HBeAg-negative hepatitis B infection. Six months after the end of a 4- to 15-month course of interferon therapy, a sustained response, defined as normal ALT levels and negative HBV DNA in hybridization assay, was found in 38 (49%) of the 78 patients with genotype A infection, compared with 17 (26%) of the 66 patients with genotype D infection ($p < 0.005$). Multivariate logistic regression analysis identified HBV genotypes and pretreatment ALT levels as independent predictive parameters of response to interferon ($p < 0.009$ and $p < 0.02$, respectively).

Kao et al. [21] retrospectively studied 58 Taiwanese HBeAg-positive patients with genotype B or C infection who had been treated with interferon alpha for 24 weeks. The patients with genotype C infection had a higher serum ALT level than patients with genotype B infection. The rate of response to interferon, defined as normalization of serum ALT level and loss of HBeAg and HBV DNA 48 weeks post-treatment was 13/32 (41%) and 4/26 (15%) in genotype B and C patients, respectively. Younger age and genotype B infection were significant and independent variables for predicting the response to interferon alpha treatment ($p = 0.001$ and 0.045, respectively).

Wai et al. [22] performed a retrospective analysis of a previously reported randomized controlled trial to determine the effects of HBV genotype on the response to interferon alpha in ethnic Chinese patients with HBeAg-positive chronic hepatitis. Among the 66 patients with elevated pretreatment ALT level, an antiviral response, defined as sustained clearance of serum HBV DNA by direct spot hybridization and clearance of HBeAg at month 12, was achieved in 57% and 21% of patients treated with interferon alpha for 16 weeks ($p = 0.019$), compared with 25% and 8% of untreated controls ($p = 0.45$) with HBV genotype B and C, respectively. Multivariate analysis showed that genotype B and low pretreatment HBV DNA levels were independent predictors of antiviral response ($p = 0.001$ and 0.029, respectively).

The attachment of a polyethyleneglycol moiety to interferon alpha produces a biologically active molecule, peginterferon alpha, with a long half-life and favorable pharmacokinetics. Janssen et al. [23] reported the results of a multicenter, randomized controlled trial of peginterferon alpha for HBeAg-positive chronic hepatitis B. For 266 patients (90 with genotype A, 23 with genotype B, 39 with genotype C, 103 with genotype D, and 11 with other types), 52-week treatment regimen was started with weekly doses of 100 µg peginterferon alpha-2b, and then during weeks 32 to 52, the dose of peginterferon was reduced to 50 µg per week. Although patients were randomly assigned peginterferon monotherapy or combination therapy with lamivudine, the rate of HBeAg loss 26 weeks post-treatment was similar between the two groups (49/136 [36%] vs 46/130 [35%]; $p = 0.91$). There was a significant difference in the sustained loss of HBeAg according to HBV genotype by univariate analysis ($p = 0.01$); 42 (47%) with genotype A and 10 (44%) with genotype B had sustainedly lost HBeAg, compared with 11 (28%) with genotype C and 26 (25%) with genotype D.

These results indicate that HBV genotypes D and C are associated with a lower rate of favorable response to interferon alpha therapy than genotypes A and B, respectively.

HBV GENOTYPES AND RESPONSE TO LAMIVUDINE

In a study conducted in Germany, Zöllner et al. [24] found that among 26 consecutive patients with chronic hepatitis B (20 were HBeAg-positive and 6 were HBeAg-negative), drug-resistant mutant variants developed in 7 of 13 carriers with *adw* and 1 of 13 carriers with *ayw* during 3 to 31 months of lamivudine treatment; the risk of lamivudine resistance was significantly higher in *adw* carriers than in *ayw* carriers ($p = 0.03$). In Germany, subtype *adw* corresponds to genotype A, and subtype *ayw* corresponds to genotype D. Zöllner et al.

[25] further studied the mutational pattern of the HBV polymerase gene in 41 patients during selection of lamivudine-resistant strains. Twenty-six patients (63%) carried resistant HBV genotype A and 15 patients (37%) carried resistant HBV genotype D. The rate of mutations from methionine to isoleucine at rt204 was significantly higher for genotype D (67%) compared with genotype A (19%), whereas mutations from methionine to valine at rt204 prevailed in genotype A (81% in genotype A vs 33% in genotype D; $p = 0.006$).

Buti et al. [26], however, reported that the proportion of lamivudine-resistant mutations did not differ between HBV genotypes A and D during longer term observation. Among the 27 patients in Spain with chronic hepatitis B (8 HBeAg-positive and 19 anti-HBe-positive) receiving lamivudine for a mean period of 24 months (range, 12–36 months), 16 had genotype A HBV (equivalent to *adw*) and 11 had genotype D (equivalent to *ayw*). At year 1 of therapy, the lamivudine-resistant variants were found more often in genotype A patients than in genotype D patients (7/16 [43%] vs 2/11 [18%]), but the difference was not significant. At year 2, the drug-resistant variants were found in 8 (53%) of the 15 genotype A patients and in 5 (55%) of the 9 genotype D patients, and at year 3, in 3 (75%) of the 4 genotype A and genotype D patients. The Kaplan–Meier estimate for the median time to lamivudine resistance in all 27 patients was 24 months, independent of HBV genotype.

Kao et al. [27] enrolled 31 consecutive Taiwanese patients with HBeAg who had been treated with lamivudine. During the study period, which ranged from 6 to 30 months, 3 (23%) of the 13 patients with genotype B infection and 2 (11%) of the 18 patients with genotype C infection had seroconversion to anti-HBe. Lamivudine-resistant mutant variants occurred in 2 (15%) patients from the genotype B group and 4 (22%) from the genotype C group ($p = 0.9$) after a mean duration of 9 months (range, 8–13 months).

In another study conducted in Taiwan, Chien et al. [28] examined the determinants of sustained HBeAg seroconversion after discontinuation of lamivudine. In a total of 82 HBeAg-positive patients who had achieved a complete response (defined as HBeAg seroconversion with HBV DNA seroclearance by hybrid capture assay and normal ALT levels) to a mean period of 16 months (range, 3–55 months) lamivudine therapy was followed-up. In 38 (61%) of the 62 patients with genotype B and 5 (25%) of the 20 patients with genotype C, complete response was sustained for 12 months after the end of therapy. The categorical analysis showed that patients with genotype B, age ≤ 36 years, and additional treatment over 8 months after HBeAg seroconversion had a higher sustained response to lamivudine.

Akuta et al. [29] determined the cumulative rate of emergence of drug-resistant mutant variants in 213 Japanese patients with chronic hepatitis B (108 were HBeAg-positive and 105 were HBeAg-negative) who had been treated with lamivudine for more than 1 year. Eight (3.8%) patients were infected with genotype A, 20 (9.4%) patients with genotype B, and 185 (86.9%) patients with genotype C. The emergence rate of lamivudine resistance was independent of the genotype, but was significantly higher in the B*a* subgroup of HBV (66.7% at 2 years) than in the B*j* subgroup (7.1% at 2 years, $p < 0.05$). In patients with genotype C infection, lamivudine-resistant variants emerged significantly more often in the HBeAg-positive state than in the HBeAg-negative state ($p < 0.05$). In contrast, the emergence rate was not associated with HBeAg status in patients with genotype B infection.

In summary, a study conducted in Germany suggested that the rate of emergence of resistance to lamivudine was higher in patients with HBV genotype A infection than in patients with genotype D infection. However, this result has not been confirmed by a longer term study. There is no difference between patients with genotype B and C for the risk of lamivudine resistance. In patients with genotype B infection, the virological response is better during lamivudine therapy, and is also more durable after the discontinuation of therapy than in patients with genotype C infection.

HBV GENOTYPES AND RESPONSE TO ADEFOVIR DIPIVOXIL AND NEW NUCLEOTIDE ANALOGUES

Westland et al. [30] examined HBV genotypes in patients with chronic hepatitis B who had been enrolled in 2 multinational phase III studies of adefovir dipivoxil. In the 269 patients (43 with genotype A, 52 with genotype B, 71 with genotype C, 96 with genotype D, and 7 with other types) who received 10 mg adefovir dipivoxil for 48 weeks, potent reductions in serum HBV DNA were observed regardless of HBV genotype, HBeAg status, or race; similarly, there was no statistical difference in HBeAg seroconversion rates between genotypes in these patients.

To date, the impacts of HBV genotype on the therapeutic response and emergence of drug-resistant variants in patients receiving entecavir or other nucleotide analogues at the stage of clinical trials have not yet been reported.

CONCLUSIONS

Differences in response to therapy with antiviral agents, including interferon alpha and lamivudine, do exist among different HBV genotypes. Genotype determination could be helpful for predicting the outcome of therapy in patients with chronic hepatitis B. However, we have to be aware that the genotype is not the only factor that determines whether a patient should be treated. In Asian countries, for example, the most prevalent genotype, genotype C, is associated with progressive liver disease; spontaneous remission of HBV infection occurs rarely in patients who chronically carry this

genotype [18]. Patients with genotype C often need to be treated with an antiviral agent, such as interferon alpha or lamivudine (which are currently available), even though the therapeutic response is expected to be poor. Further studies are needed to clarify the efficacy of new antiviral agents in patients with various genotypes.

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Timing of interferon therapy and sources of infection in patients with acute hepatitis C

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Abstract

Background/Aims: Controversy over the selection of patients and optimum therapeutic method for acute hepatitis C has continued. The aims of this study were to investigate the source of infection, and to evaluate the timing of interferon (IFN) therapy in patients with acute hepatitis C in Japan.
Methods: The records of 102 patients from 12 facilities in Japan who developed acute hepatitis C after 1990 were investigated. In the patients treated with IFN, we performed multivariate analysis to investigate factors related to sustained virological response (SVR).
Results: Medical procedure was the most common source of infection, accounting for 32.4% in the 102 patients (33/102). Of 81 patients treated with IFN, 71 patients were followed after IFN therapy, and 57/71 (80.3%) had SVR. The SVR rate was significantly higher in patients treated with IFN within 24 weeks from onset of symptoms than the SVR rate in those treated after 25 weeks ($P = 0.0016$). Multivariate analysis revealed that only the duration between onset of symptoms and initiation of IFN therapy (within 24 weeks) was related to SVR.

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Abbreviations: HCV, hepatitis C virus; IFN, interferon; ALT, alanine aminotransferase; SVR, sustained virological response; Peg-IFN, pegylated interferon
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Conclusions: Our multicenter cooperative survey revealed that medical procedure was the most frequent source of infection in acute hepatitis C. As concerns the therapy, interferon treatment should be initiated within 24 weeks after onset of symptoms.

Keywords: Hepatitis C virus (HCV); Acute hepatitis; Medical procedure; Interferon

1. Introduction

There are about 170 million people infected with the hepatitis C virus (HCV) worldwide [1], and the infection progresses to hepatic cirrhosis in 10–30% [1,2]. Since patients often lack subjective symptoms even in acute hepatitis C [3], infection is often realized by patients when the pathology progresses to hepatic cirrhosis and hepatocellular carcinoma. There are a variety of sources of infection, such as medical procedure, intravenous drug use, and sexual behavior [4,5]. In addition, vertical transmission of HCV has been reported, and it seems that maternal viral load is significant for infection to fetus [6]. On the other hand, as a therapy for acute hepatitis C, interferon (IFN) administration has been established to be effective [4,5,7–13].

Although the initial prevention of hepatitis C virus (HCV) infection is ideal, the most effective method of preventing progression to the chronic hepatitis C is still controversial in the acute phase. In Japan, the development of acute hepatitis C due to blood transfusion has markedly decreased after introduction of the HCV antibody test for screening of blood donors [14]. However, infection from intravenous (i.v.) drug use and incidences due to accidental contamination of medical staff are still important problems [15,16]. Investigation for the sources of infection in acute hepatitis C is very important for the prevention. In this study, we investigated a national survey on the route of infection of acute hepatitis C and the therapeutic effectiveness according to the timing of IFN therapy. This survey consists of the largest number of case reports and may reflect the current situation of acute hepatitis C in Japan.

2. Patients and methods

2.1. Patients

A retrospective study was performed in patients of 12 facilities nationwide who developed acute hepatitis C after 1990. The total number of patients at the facilities was 102. Informed written consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Age, gender, source of infection, HCV serotype or genotype, HCV-RNA level, histology of liver biopsy, fluctuation in alanine aminotransferase (ALT) level, presence or absence of IFN therapy, course when not treated with IFN, duration between onset of symptoms and IFN therapy, type of IFN, total dose of IFN, administration method, total duration of administration, and therapeutic results were investigated in each patient.

2.2. Diagnosis of acute hepatitis C

The diagnostic criteria of acute hepatitis C were HCV-RNA detectable at the time of an elevated ALT level, followed by development conversion of HCV antibody. Patients in whom HCV antibody was already positive at the onset were excluded.

2.3. Natural course

In patients who followed the natural course without any treatments, the chronic hepatitis was defined as persistence of HCV-RNA positivity for 6 months or longer, and resolution was defined as a disappearance of serum HCV-RNA within 6 months followed by persistent negativity for 6 months or longer.

2.4. Definition of fluctuation of ALT

In patients diagnosed with acute hepatitis C, when one peak of the serum ALT level was observed, the fluctuation was designated as monophasic, and when two or more peaks were observed, the fluctuation was designated as bi- or multiphasic.

2.5. Serologic tests

Anti-HCV antibody was determined using a second-generation or third-generation enzyme-linked immunosorbent assay (Ortho Diagnostics Systems, Tokyo, Japan). Hepatitis C virus RNA was quantified by using the bDNA signal amplification assay (Chiron Corp.) or the Cobas Amplicor HCV Monitor test ver1.0 or 2.0 (Roche Diagnostic Systems, Tokyo, Japan). The data were represented as Meq/ml, K copies/ml, and KIU/ml, respectively. Detection of HCV-RNA to determine the response of IFN treatment was used by Amplicor HCV (Roche Diagnostics K.K., Japan). Hepatitis C virus serotype was determined using the genotyping enzyme-linked immunosorbent assay (International Reagents Corporation, Tokyo, Japan) to be type 1 or 2 [17].

2.6. IFN therapy

For IFN, IFN-α (natural form, gene recombinant, or consensus IFN), or IFN-β was used (Table 4). No concurrent treatment with IFN and ribavirin was administered to any patient. Among patients treated with IFN, the sustained

virological response (SVR) was defined undetectable HCV-RNA in serum at least 6 months after cessation of therapy. Non-response was defined as detectable HCV-RNA for 6 months after cessation of therapy.

2.7. Statistical analysis

Data were expressed as the mean ± standard deviation for continuous variables and as counts for categorical variables. The results were compared using the Chi-square test, Fisher's exact probability test, or Mann–Whitney *U*-test, depending upon the type of data analysed. Logistic regression was used to analyse the factors contributing to SVR with IFN therapy. *P* values <0.05 were considered significant. Statistical analyses were performed by using Stat View software (version 5.0; SAS Institute Inc., Cary, NC).

3. Results

3.1. Patient characteristics

The baseline characteristics of the 102 patients in this study are shown in Table 1. The distribution of patients by gender and age is shown in Table 2.

3.2. Natural course

The natural course of the disease was followed in 21 patients, and the course could be followed to the outcome in 18 patients (the prognosis was unknown in three patients) (Table 3). The disease progressed to chronic hepatitis C in 61.1% of the patients and resolved spontaneously in 38.9% of the patients. The age and the fluctuation pattern of the ALT level were significantly different between the two groups. As for gender, serum HCV-RNA level, and serogroup, no correlation with spontaneous resolution or chronic hepatitis C was observed.

Table 1
Base-line characteristics of 102 patients

Age	38.6 ± 16.2 (16–84)
Male/female (mean age)	46 (39.2 ± 16.0)/56 (38.2 ± 16.5)
Source of infection (%)	
Medical procedure	33 (32.4)
Accidental needle stick	21 (20.6)
Sexual behavior	8 (7.8)
Drug abuse	6 (5.9)
Tattoo	3 (2.9)
Unknown	31(30.4)
Viral load (high[a]/low/N.D.)	46/45/11
HCVserotype(1/2/N.D.)	54/23/25
IFN/without IFN	81/21

N.D., not determined; IFN, interferon. Details of the routes in medical procedure: surgery 14, blood transfusion 5, endoscopy 3, intravenous injection 4, invasive procedure 3, dental therapy 3, dialysis 1.
[a] Viral load (high): more than 100 KIU/ml or 1 Meq/ml.

3.3. IFN therapy

Table 4 shows the backgrounds of the 81 patients treated with IFN. Of 71 patients in whom the effect was clarified,

Table 2
Distribution of patients according to gender and age

Age (years)	Number of patients					
	Medical procedure (M/F)	Accidental needlestick (M/F)	Sexual behavior (M/F)	Drug abuse (M/F)	Tattoo (M/F)	Unknown (M/F)
<19	0/1	0/0	0/0	0/1	0/0	0/1
20–29	5/1	3/8	1/3	2/1	3/0	2/6
30–39	4/3	3/3	2/1	0/1	0/0	3/3
40–49	2/4	0/4	1/0	0/1	0/0	2/3
50–59	4/3	0/0	0/0	0/0	0/0	2/3
60–69	4/1	0/0	0/0	0/0	0/0	2/0
70–79	0/0	0/0	0/0	0/0	0/0	1/1
>80	0/1	0/0	0/0	0/0	0/0	0/2
Total	19/14	6/15	4/4	2/4	3/0	12/19

M, male, F, female.

Table 3
Base-line characteristics of 18 untreated patients

	Resolved group (seven cases)	Chronic group (11 cases)	*P* value
Age	64.4 ± 15.2	45.6 ± 14.3	0.0331[a]
Gender (male/female)	2/5	4/7	>0.9999
HCV RNA level (high[b]/low/N.D.)	2/4/1	6/4/1	0.6084
Serogroup (1/2/N.D.)	4/0/3	4/2/5	0.4667
Fluctuation of ALT level (monophasic/bi- or multiphasic/N.D.)	5/0/2	0/8/3	0.0008[a]

N.D., not determined; ALT, alanine aminotransferase. Fluctuation of ALT level: monophasic; one peak of the serum ALT was observed, bi- or multiphasic; two or more peaks of the serum ALT were observed (N.D. was excluded from statistical comparisons).
[a] Statistically significant.
[b] Viral load (high): more than 100 KIU/ml or 1 Meq/ml.