patients infected with HBV and to determine the effectiveness of IFN therapy in suppressing the rate of recurrence of HBV-related HCC in these patients. For this purpose, we compared young noncirrhotic patients with HCC with older noncirrhotic patients with HCC and also older cirrhotic patients with HCC.

Patients and methods

Patients

From August 1978 to October 2002, a total of 187 patients with chronic hepatitis B were treated for HCC at Toranomon Hospital, Tokyo, Japan. They included 155 males and 32 females, aged 10 to 80 years, with a median age of 52 years. Hepatitis B e antigen (HBeAg) was positive in 56 (30%) and anti-HBe in 98 (52%). All patients were negative for anti-HCV antibody. In terms of the incidence of HBV-related HCC by underlying liver tissue, HCC developed from liver cirrhosis cases in 166 (89%) of 187 cases and from chronic hepatitis cases in 21 (11%) cases.

Among these patients, all four patients who were less than 30 years of age had no cirrhotic liver disease. These four patients were labeled as cases of young adult HCC. They consisted of two males and two females ranging in age from 10 to 26 years with a median age of 22.5 years at the time of diagnosis of HCC.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital, and a signed consent form was obtained from the subjects or their parents/relatives.

Blood tests

All four patients were positive for HBsAg as determined by hemagglutination, using commercially available kits (MyCell; Institute of Immunology, Tokyo, Japan), and HBeAg and anti-HBe as measured by radioimmunoassay (RIA; Abbot Diagnostics, Chicago, IL, USA). HBV-DNA was measured by branched DNA signal amplification technology (bDNA assay) (Chiron, Emeryville, CA, USA), transcription-mediated amplification and hybridization protect assay (TMA) (Chugai Diagnostics, Tokyo, Japan),[15] and Cobas Amplicor HBV Monitor Test (Amplicor) (Roche Diagnostics, Branchburg, NJ, USA). Genotyping of HBV was performed by an enzyme-linked immunosorbent assay (ELISA) kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) using monoclonal antibodies for the genotype-specific epitopes in the pre S2 region product.[16] Subgrouping of genotype B, Ba and Bj, was performed based on the method described by Sugauchi et al.[17] At the time of the diagnosis of HCC, background liver function was evaluated by indocyanine green retention rate at 15 min (ICG R15).

Liver histology

Liver tissues were obtained by surgical resection in one patient and by ultrasound-guided biopsies in two patients. Histological staging of the nontumorous liver tissue was based on the classification proposed by Desmet et al.[18] The remaining single patient did not undergo histopathological examination.

Family history

Information about the family history of HBV-related liver disease and the presence of HBsAg was obtained by asking patients and their family members about their past history.

Results

Incidence of HCC by age

Figure 1 shows the incidence of HBV-related HCC by age at diagnosis. For all 187 patients, the peak incidence of HCC associated with liver cirrhosis occurred in the 50–59 years age group while the peak incidence of HCC associated with chronic hepatitis occurred in the 40–49 years age group. Interestingly, all cases with HCC in the 10–19 and 20–29 years age groups had chronic hepatitis.

Comparison of clinicopathological profiles according to age and underlying liver tissue

We classified 187 HBV-related HCC cases into three categories depending on patient age (under or above 30

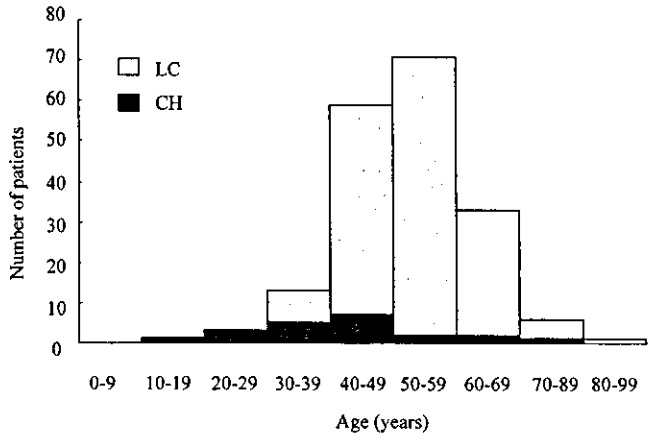

Fig. 1. Incidence of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) by age. *LC*, liver cirrhosis; *CH*, chronic hepatitis

Table 1. Comparison of profiles of hepatocellular carcinoma (HCC) patients without cirrhosis under 30 years of age, and those over 30 years of age with HCC and without cirrhosis or HCC with cirrhosis

	<30 years Cirrhosis (−) $n = 4$	≥30 years Cirrhosis (−) $n = 17$	≥30 years Cirrhosis (+) $n = 166$
Age (years)[a]	22 (10–26)	45 (36–45)	52 (32–80)
Sex (M:F)	2:2	15:2	138:28
eAg/eAb	0/4	3/12 (two sides, 2)	53/86 (two sides, 21) (unknown, 2)
HBV genotype A:B:C:D(E)	0:2:2:0	0:2:12:1 (not tested, 2)	0:6:148:0 (undetectable, 3) (not tested, 9)
Maternal HBV-related liver disease (%)	4 (100)	7 (41)	46 (28)

M, male; F, female; eAg, HBeAg; eAb, anti-HBe; HBV, hepatitis B virus
[a] Median value (range)

years), and presence or absence of liver cirrhosis. Table 1 provides a comparison of patient profiles. The proportion of females among the 4 young cases with young adult HCC (50%) was relatively high in comparison with the other two groups. Similarly, for the HBsAg genotype, the proportion of patients with genotype B was relatively higher in the test cases than in the other two groups.

In terms of the state of HBeAg/anti-HBe at the time of the diagnosis of HCC, all 4 test patients were positive for anti-HBe, whereas the proportions of cases positive for anti-HBe were 12 of 17 cases (71%) in the group without cirrhosis over 30 years of age and 86 of 166 cases (52%) in the group with cirrhosis over 30 years of age.

With regard to the family history, the mothers of all patients without cirrhosis under 30 years of age had HBV-related liver disease, including death from HCC in one, liver cirrhosis in two, and one asymptomatic carrier (Fig. 2). However, the incidence of maternal HBV-related liver disease in other groups tended to be lower; 7 of 17 cases (41%) in the group without cirrhosis over 30 years of age and 46 of 166 cases (28%) in the group with cirrhosis aged over 30 years of age.

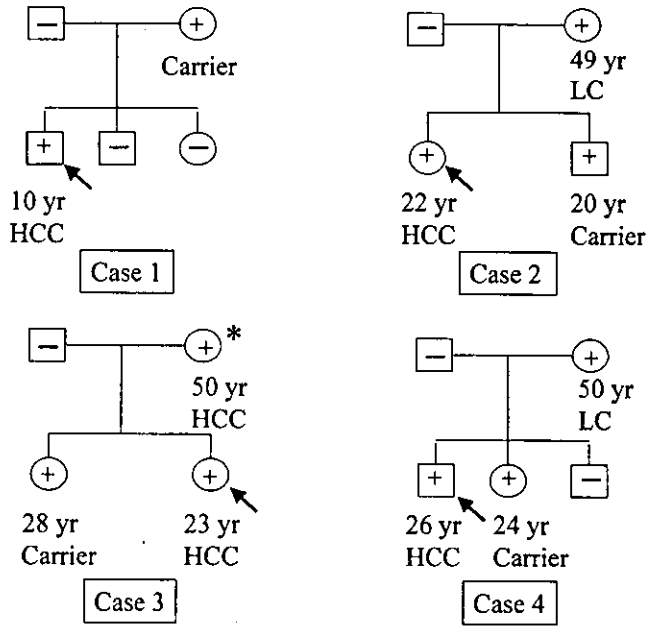

Fig. 2. Pedigree of four cases: +, positive HBsAg; −, negative HBsAg; *arrows*, HCC cases; *asterisk*, death

Clinical background of the four cases of young adult HCC

Table 2 summarizes the background characteristics of the four young adult HCC patients. Serum HBeAg was negative and anti-HBe was positive in all four cases. Distribution of HBV genotypes was two cases (50%) for genotype Bj and two cases (50%) for genotype C. HBV-DNA (b DNA assay) was measured in only one case (case 2) at the time of diagnosis of HCC, and serum HBV level was less than 0.7 mEq/ml. HBV-DNA was not measured in the other three cases because they were first diagnosed with HCC at other hospitals, and thus the HB viral loads of these patients were not clear. Laboratory tests revealed almost normal liver function based on the results of the ICG R15 test in all four patients.

Histopathological examination of the nontumorous liver was conducted in three of the four cases. In one case (case 1), there was almost normal architecture and

Table 2. Background of four cases with cirrhosis-unrelated HCC

Case	Sex	Age (years)	eAg/eAb	HBV genotype	Viral load bDNA assay (mEq/ml)	Histology	ICG (%)
1	M	10	−/+	Bj	NT	F0/A0	8
2	F	22	−/+	C	<0.7	NT	5
3	F	23	−/+	C	NT	F0/A0	6
4	M	26	−/+	Bj	NT	F1/A1	7

+, positive; −, negative; NT, not tested at the time of diagnosis of HCC; ICG, indocyanine green

Table 3. Clinical course of survival cases after HCC

Case	Treatment	Histological differentiation in last HCC	Viral load bDNA assay[a] (mEq/ml)	Intervals of interferon (IFN) administration[b] (years)	Survival period[c] (years)
1	TAE ×3 Operation ×3	Moderately	<0.7	3.3	>4.2
3	Operation ×2	Moderately–poorly	<0.7	3.1	>4.5
4	Operation ×2 TAE ×3	Moderately	<0.7	3.2	>3.5

TAE, transcatheter arterial embolization
[a] At the initiation of IFN therapy
[b] After completion of treatment for HCC, patients were administered 3 MU of IFN-α-2a twice a week
[c] Survival periods indicate time from the completion of treatment for HCC

no inflammation apart from mild irregularities in the size of hepatic cell nuclei. Case 3 also showed a normal liver architecture, very mild portal inflammation, and sparse focal necrosis in the lobular region. Case 4 showed a slight expansion and inflammation of the portal region and was diagnosed as chronic hepatitis (F1/A1).

Clinical course after HCC and efficacy of IFN therapy

At the time of diagnosis of HCC, case 2 had a large hepatoma that extended into both liver lobes with portal invasion, as evident by imaging studies including ultrasonography (US), computerized tomography (CT), and abdominal angiography. This patient underwent transcatheter arterial embolization (TAE) twice, but she died 4 months after the discovery of the tumor. Table 3 outlines the clinical course of the three surviving patients (cases 1, 3, and 4) after the diagnosis of HCC. Because they had recurrent HCC, they underwent surgical resection and TAE for HCC more than once. The histopathological differentiation determined for the last HCCs was not greater than moderately differentiated HCC in all three. After radical operation for HCC, each patient was treated with 3 million units (MU) of IFN-α-2a, twice a week, with the aim of preventing recurrence of HCC. Figure 3 shows the clinical course after the last therapy for HCC and the initiation of IFN therapy in these three cases. All patients showed stable low viral loads and normal liver function. As part of the follow-up monitoring for recurrence, imaging studies were performed every 3–4 months by using US or CT. These studies did not reveal recurrence of HCC during more than 3 years after the initiation of IFN therapy.

Discussion

Chronic infection with HBV is associated with increased risk for the development of cirrhosis and HCC. Based on the report of Ikeda et al.,[6] the incidence of progression of HBV-related chronic hepatitis to cirrhosis is 2.2% per year, whereas the incidence of carcinogenesis is 0.5% per year during the first 10 years and 1% thereafter. Using multivariate analysis, we also showed that the severity of the fibrotic stage in HBV-related chronic hepatitis was not associated with the development of HCC.[6] Another clinicopathological study of HCCs in chronic HBV carriers revealed that about 20%–50% of such patients do not have accompanying cirrhosis.[8]

With regard to the replicative state of HBV, after HBeAg to anti-HBe seroconversion associated with

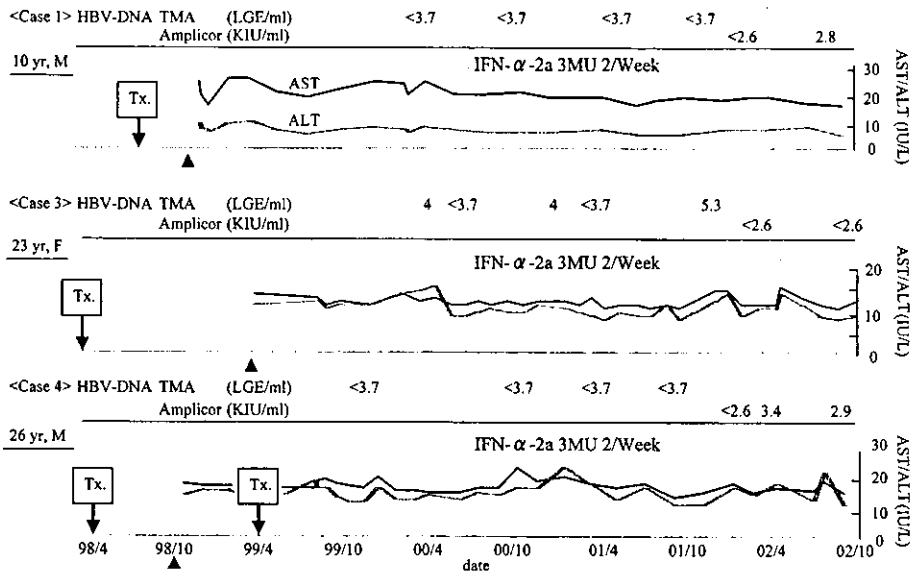

Fig. 3. Clinical course after radical surgery for last HCC. *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *Tx.*, therapy for HCC; *arrowheads*, time of first visit to our hospital

loss of viral replication and remission of disease activity, patients are usually asymptomatic and liver disease is inactive; however, some patients may progress to HCC.[19] In HCC, previous studies showed HBV DNA integration into the cellular genomic DNA.[4,10,11] Although the role of the integration in hepatocarcinogenesis is not clear at present, Dandri et al.[20] speculated that disturbances of repair of DNA damage, which occurred either on cellular or viral DNA with consecutive activation of recombination events, are responsible for the increased viral integration frequency.

HCC with HBV infection is sometimes found in very young patients, and the majorities of such patients have no cirrhosis and are negative for HBeAg but positive for anti-HBe, suggesting a nonreplicative state of HBV.[9] In these cases, HBV DNA integration is common. Because the high frequency of HBV DNA integration occurs at early stages of chronic infection, it has been suggested that the latent period of HCC after HBV infection is much shorter than in adult patients.[10-12]

In the present study, the characteristics of HBV-related HCC in young adult patients without cirrhosis were similar to those described in previous reports.[9-12] All cases were negative for HBeAg and positive for anti-HBe; the histopathological stage of nontumorous liver tissue was F0 or F1, representing mild hepatitis, at the time of diagnosis of HCC. Interestingly, family history of maternal transmission of hepatitis B virus was noted in all four cases. Unfortunately, we did not investigate the integration of HBV DNA into the tumorous and nontumorous liver tissues of these cases. Considering the above features and results of previous studies, we speculate that the development of HCC in our cases at a young age might be due to HBV DNA integration into the cellular genomic DNA during fetal life. It is very difficult to clearly explain why these four patients developed HCC at such a young age without cirrhosis. Although we cannot demonstrate any solid evidence, we hypothesize that at least in some cases, the development of HCC at a young age is related to transplacental transmission of HBV during intrauterine life from the infected mother and HBV DNA integration into the cellular genomic DNA during fetal life. Due to the HBV DNA integration in earlier stages of chronic infection, HCC developed within a short period at young age, as speculated by Chang et al.[9,11,12] and Goto et al.[10]

Previous studies reported a higher incidence of HCC in males than in females and that the rate of progression of HBV infection was faster in males than in females.[19] On the other hand, in terms of HBV genotypes, none of the reported HCC patients younger than 35 years of age had genotype B in Japan (genotype Bj), different from genotype B in Taiwan (genotype Ba), and genotype Bj is regarded to be associated with a relatively good prognosis.[21,22] Our results are different from those of the above reports; two of four patients are female and genotype B of HBV genotype, especially genotype Bj accounts for half of our patients. Furthermore, despite the relatively good prognosis reported in the these studies, our cases developed HCC early in life. Although the number of the young adult HCC was relatively small, these factors may indicate that HCC in young adults is a little different from HCC among elder patients.

HBV vaccine became available in 1980, and since 1986, initiatives have been taken to prevent mother-to-child transmission of HBV in Japan. Furthermore, since 1996, HBV vaccine has also been administered to

babies born to HBsAg-positive mothers without HBeAg. While the protection efficacy is high, 5%–10% of infants with intrauterine HBV infection caused by transplacental transmission develop the infection in spite of receiving HBV vaccine.[23] Although HBV vaccine against vertical transmission is clearly effective and may reduce the incidence of liver cirrhosis and HCC, HBV carriers who cannot be protected by HBV vaccine can develop HBV infection by transplacental transmission; in other words, the HBV DNA is already integrated into the host DNA at birth. These children should be carefully monitored for the development of HCC.

In a pilot study, we reported that long-term intermittent IFN therapy successfully reduced hepatocellular carcinogenesis in patients with HBV-related cirrhosis.[13] Our virological study showed that the role of IFN therapy from the point of view of cancer prevention was much more significant in patients with HBV DNA concentration ≧10 mEq, and hence we considered that IFN could suppress carcinogenesis in HBV-related cirrhosis through the suppression of HBV replication. In our study, all three surviving cases suffered repeated recurrence of HCC, and thus were considered a high-risk group for hepatocellular carcinogenesis. However, after the radical operation for the last HCC, and due to long-term intermittent IFN therapy, they showed good prognosis, survival, and no recurrence of more than 3 years, and also showed persistently low viral loads (by bDNA assay) and normal liver function during follow-up. Based on these observations, it seems that long-term intermittent IFN therapy could prevent the recurrence of HBV-related HCC and act as an antitumor agent.

Although we did not confirm the integration of HBV DNA into the cellular genomic DNA of these patients and have no evidence of transplacental transmission of HBV, these mechanisms remain speculative at this stage and further studies of a larger population sample are warranted.

In conclusion, we have demonstrated in the present study that (1) four young adult HCC patients were positive for anti-HBe, were free of liver cirrhosis, and in all the route of infection seemed to be mother-to-infant transmission. Transplacental transmission of HBV and HBV DNA integration into the cellular genomic DNA during fetal life is a possible explanation of HBV-related hepatocarcinogenesis in young adults; and (2) long-term IFN therapy might be useful for prevention of tumor recurrence after radical operation for HBV-related HCC. To confirm these potential mechanisms, further long-term follow-up studies of large number of patients are necessary. Furthermore, prospective studies should examine the development of HCC in HBV carrier children who could not be protected by HBV vaccine, to confirm the presence of HBV DNA integration in cellular genomic DNA and also to investigate the host factors associated with early hepatocarcinogenesis, including oncogenes and suppressor oncogenes.

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Infection with hepatitis B virus genotype A in Tokyo, Japan during 1976 through 2001

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Background. Because genotype A of hepatitis B virus (HBV) is not indigenous, there have been only few data on infection with it in Japan. ***Methods.*** We examined clinical and virological features of the 66 Japanese patients who admitted Toranomon Hospital in Tokyo, Japan, between 1976 and 2001, who were found to have HBV/A infection. HBV genotype A was classified into subtype A (European type) and A′ (South African type) by phylogenetic analysis of the preS1 and preS2 regions, and the S gene sequences. ***Results.*** Of the 66 patients infected with HBV/A, 14 (21%) were asymptomatic carriers, 26 (39%) presented with acute hepatitis, 22 (33%) with chronic hepatitis, and 4 (6%) with liver cirrhosis. HBV/A infection persisted for more than 6 months in 5 of the 26 (19%) patients with acute hepatitis. The frequency of acute hepatitis in patients infected with HBV/A was higher after than before 1991 (2/22 [9%] vs 24/44 [55%]; $P < 0.0001$). The frequency of nucleotide 1858 of T was higher in asymptomatic carriers than in patients with acute hepatitis in whom infection was resolved (5/14 [36%] vs 0/21 [0%]; $P = 0.008$). Of the 57 patients for whom subtypes of genotype A were determined, subtype A was identified in 53 (93%) and subtype A′ in only 4 (7%). All patients infected with subtype A′ were persistently infected with HBV. ***Conclusions.*** HBV/A infection has become more frequent during recent years, predominantly presenting as acute hepatitis, and subtype A′ is uncommon in the Tokyo metropolitan area.

Key words: genotypes, hepatitis B virus, hepatitis B e antigen, subtypes

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Introduction

Hepatitis B virus (HBV) is classified into seven genotypes by a sequence divergence in the entire genome of at least 8%, and named by capital alphabet letters from A to G.[1–3] HBV genotypes have distinct geographic distributions.[4,5] HBV Genotype A is prevalent in Northwestern Europe, North America, and South Africa, and was not reported in any of the Asian HBV genomes isolated until 1990.[1,6]

In 1997, Bowyer et al.[7] identified a unique subgroup of genotype A, based on comparison of the large S gene sequence, and named it A′. Of South African HBV/A isolates, 59% were classified into subgroup A′ and clustered to form a segment discrete from the original genotype A isolates. Amino-acid differences that set A′ isolates apart from the rest of group A tend to cluster in the pre-S2 region (amino acids 7, 10, 32, 35, 47, 48, 53, and 54), with a few changes in the major surface antigen (amino-acid sites 207 and 209). HBV isolates of subgroup A′ were separated from those in Northwestern Europe genotype A by sequence differences ranging from 4.1% to 6.2% in the preS2/S region,[7] as well as by phylogenetic differences in the entire genomic sequence.[8]

To our knowledge, there are no large-scale studies on Japanese patients infected with HBV of genotype A or subgroup A′ with clinical and virological characterizations. In the present study, we examined 68 Japanese patients infected with HBV/A who visited Toranomon Hospital in the Tokyo metropolitan area during 1976 through 2001, and we examined their clinicopathological characteristics and virological features. Further, HBV/A isolates were classified into subtypes A and A′ by phylogenetic analysis. The nucleotide (nt) 1858 of T or C was determined, also, which influences the development of the precore stop-codon mutation at nt from G to A.[9,10]

Patients and methods

Patients

Among the patients who admitted the Department of Gastroenterology, Toranomon Hosital, in metropolitan Tokyo during 1976 through 2001, 2022 were infected with HBV and received liver biopsy. Genotypes of HBV were determined in these patients, and genotype A was detected in 68 (3.4%), B in 239 (11.8%), C in 1649 (81.6%), D in 5 (0.2), and F in 2 (0.1%) of them; HBV isolates from the remaining 59 (2.9%) patients were untypeable. The 68 patients infected with HBV/A were examined medically for the diagnosis of liver disease, and HBV DNA in their sera were studied virologically. The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital, and an informed consent was obtained from each of the patients.

These 68 patients infected with HBV/A were classified into four clinical groups. An asymptomatic carrier state was diagnosed in 14 (21%) patients. They had serum alanine aminotransferase (ALT) levels consistently within the normal range (≤50 IU/l) and ultrasonographic findings of a "normal liver" or "fatty liver" at the initial visit. Chronic hepatitis was diagnosed in 22 (32%) patients, based on histopathological examination of liver biopsy specimens obtained under laparoscopy. Four (6%) patients had already developed liver cirrhosis that was diagnosed by findings on laparoscopy and imaging modalities, including ultrasonography and computed tomography.

The remaining 28 (41%) patients were diagnosed with acute hepatitis. They had all contracted HBV infection in adulthood, and possessed antibody to hepatitis B core antigen of IgM class in high titers (2.2–11.7) in serum for the diagnosis of acute hepatitis B. De-novo HBV infection in them was confirmed by the lack of hepatitis B surface antigen (HBsAg) at health checkups they had received before they came down with acute hepatitis.

Serum markers of HBV infection

HBsAg was determined by hemagglutination (MyCell; Institute of Immunology, Tokyo, Japan) and radioimmunoassay (AUSRIA II-125; Dinabot, Tokyo, Japan). Hepatitis B e antigen (HBeAg) and the corresponding antibody (anti-HBe) were determined by radioimmunoassay (AUSRIA-II 125; Dinabot). HBV DNA was determined by transcription-mediated amplification and hybridization assay (TMA; Chugai Diagnostics, Tokyo, Japan) and the results were expressed as log genome equivalents (LGE) per milliliter of serum, with detection limits ranging from 3.7 LGE/ml (corresponding to 5000 copies/ml) to 8.7 LGE/ml. Genotypes of HBV were determined, in sera from the patients at presentation, by commercial enzyme-linked immunosorbent assay (ELISA) kits (HBV Genotype EIA; Institute of Immunology), using monoclonal antibodies against epitopes on the pre-S2-region products.[11,12] Serotypes of HBsAg were determined by ELISA, using commercial kits (HBs Antigen Subtype EIA; Institute of Immunology).

Determination of subtypes A and A′ of genotype A

Nucleic acids were extracted from serum (100 µl), using the Smitest EX & R kit (Genome Science, Tokyo, Japan) and stored at −80°C. Nucleotide sequences spanning the large S gene (preS1 and preS2 regions and the S gene) were amplified in extracted nucleic acids by polymerase chain reaction (PCR) with nested primers. The first-round PCR was performed with BGF1 (sense, 5′-CTG TGG AAG GCT GGC ATT CT-3′ [nt 2757–2776]) and BGR2 (antisense, 5′-GGC AGG ATA GCC GCA TTG TG-3′ [nt 1050–1079]) primers, and the second-round PCR with PLF5Bm (sense, 5′-TGT GGA TCC TGC ACC GAA CAT GGA GAA-3′ [nt 136–162] and BR112 (Antisense, 5′-TTC CGT CGA CAT ATC CCA TGA AGT TAA GGG A-3′ [nt 163–864]), as well as BGF5 (sense, 5′-TGC GGG TCA CCA TAT TCT TG-3′ [nt 2811–2830]) and BGR6 (antisense, 5′-AGA AGT CCA CCA CGA GTC TA-3′ [nt 2831–248]) for 35 cycles each (94°C, 1 min [5 min in the first cycle]; 53°C, 2 min; and 72°C, 3 min [7 min in the last cycle]). The amplification products were run on gel electrophoresis and stained with BIG Dye (Applied Biosystems, Foster City CA, USA). Then, they were purified by QIAquick PCR purification kit (Qiagen, Hilden, Germany), and sequenced in an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequences of the large S gene in HBV DNA samples from patients were analyzed phylogenetically, along with reference sequences of subtypes A and A′,[7] by six-parameter[13] and neighbor-joining methods.[14]

Mutations in the core promoter and precore region

For determination of the wild-type or mutants in the core promoter and precore region, nucleic acids extracted from serum were amplified by PCR with nested primers. The first-round PCR was performed with BCP-F7 (sense, 5′-TGC ACT TCG CTT CAC CTC TG-3′ [nt 1580–1599]) and BCP-R8 (antisense, 5′-TAA GCG GGA GGA GTG CGA AT-3′ [nt 2295–2276]) primers, and the second-round PCR with BCP-F5 (sense, 5′-GCA TGG AAC CAC CGT GAA C-3′ [nt 1606–1625]) and BCP-R6 (antisense, 5′-ATA CAG AGC AGA GGC GGT AT-3′ [nt 2014–1995]) for 35 cycles

Table 1. Comparison of 66 patients who were infected with HBV genotype A and presented with various hepatic diseases

Features	ASC (n = 14)	AH (n = 26)	CH (n = 22)	LC (n = 4)	Differences
Male	10 (71%)	26 (100%)	19 (86%)	3 (75%)	NS
Age (years)[a]	28 (18–62)	33 (21–56)	36 (12–55)	43 (22–66)	NS
Follow-up (years)[a]	5 (0.6–15)		9 (3–20)	8 (0.3–21)	NS
Family history[b]	5 (36%)		3 (14%)		NS
Father	3 (21%)				
Mother			1 (5%)		
Siblings	2 (14%)		2 (9%)		
Transfusion	0	0	0	1 (25%)	NS
HCV RNA	0	0	1 (5%)	1 (25%)	NS

ASC, asymptomatic carriers; AH, acute hepatitis; CH, chronic hepatitis; LC, liver cirrhosis
[a]Median values are shown, with ranges in parentheses
[b]Family member with HBV-associated liver disease according to statements of patients

each (94°C, 1 min [5 min in the first cycle]; 53°C, 2 min; and 72°C, 3 min [7 min in the last cycle]). The amplification products were run on gel electrophoresis, purified, and sequenced as described above. Mutations interfering with translation or transcription of HBeAg were searched for in the precore region and core promoter. They included a G-to-A mutation at nt 1896 in the precore region (A1896) and a double mutation in the core promoter, converting codon 1762 from A to T, and codon 1764 from G to A (T1762/A1764). Also examined was nt 1858 of T or C in the precore sequence.

Statistical analysis

Nonparametric procedures were used for the analysis, including the Mann-Whitney U-test, Fisher's exact test, and Bonferroni test. A P value of less than 0.05 in two-tailed analysis was considered significant.

Results

Characteristics of patients

Table 1 compares demographic features, history of transfusion, and infection with hepatitis C virus (HCV) in the 68 patients who were infected with HBV/A and presented with various liver diseases at the first hospital visit. They were all positive for HBsAg of serotype *adw* in serum. There were no differences in sex, age, duration of follow-up, family members infected with HBV, and history of blood transfusion among them. There was only one mother positive for serum HBsAg in the patients with chronic hepatitis. Her genotype of HBV was B, different from genotype A in her son, who was diagnosed with chronic hepatitis. Homosexual contacts were reported by 11 of the 28 (39%) patients with acute hepatitis. HBV infection persisted in 4 of the 11 (36%) patients who had contracted HBV/A infection through homosexual activity. HCV RNA was detected in 1 patient with chronic hepatitis and 1 with liver cirrhosis.

Of particular note, HBV/A infection persisted for longer than 6 months in 5 of the 26 (19%) patients who presented with acute hepatitis. On liver biopsies undertaken 6–96 months after the estimated time of infection, necroinflammatory changes and fibrosis were detected in all the 5 patients for the diagnosis of chronic hepatitis B.

For the purpose of analyzing trends of liver disease that may change with time, patients were divided into two groups according to the time of the first visit to the Gastroenterology Department at Toranomon Hospital, i.e., before or after 1991, when the serological diagnosis of HCV infection had become nationwide. There were 22 patients in 1976–1990 and 44 patients in 1991–2001. Figure 1 compares the proportions of patients who presented with four categories of clinical diagnosis between the two groups of patients.

There was an apparent increase in the proportion of patients who presented with acute hepatitis in the period 1991–2001 compared to 1976–1990 (2/22 [9%] vs 24/44 [55%]; $P < 0.0001$). By contrast, the proportions of asymptomatic carriers, as well as those of patients with chronic hepatitis and liver cirrhosis, decreased after 1991 (27% vs 17%, 55% vs 22%, and 9% vs 4%, respectively; $P < 0.0001$ for all).

Many patients diagnosed as asymptomatic carriers and those diagnosed with chronic hepatitis or liver cirrhosis in 1976–1990 were less than 40 years of age. However, in 1991–2001, there were many patients diagnosed with chronic hepatitis or liver cirrhosis who were 40 years or older (Fig. 2). Thus, of asymptomatic carriers, those aged less than 40 years significantly increased in frequency from 1976–1990 to 1991–2001. In a mirror image, the frequency of patients younger than 40 years significantly decreased in those with chronic hepatitis or liver cirrhosis. Patients with acute hepatitis were excluded from the comparison, because only three (11%) of them were 40 years or older at presentation.

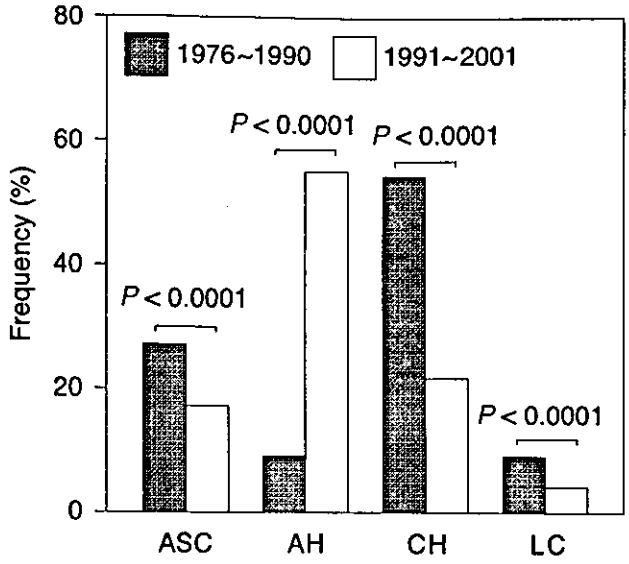

Fig. 1. Comparison of the proportions of liver diseases in patients infected with hepatitis B virus (HBV) genotype A who visited Toranomon Hospital between 1976 and 1990 and between 1991 and 2001. *ASC*, asymptomatic carriers; *AH*, acute hepatitis; *CH*, chronic hepatitis; *LC*, liver cirrhosis

Wild-types in the core promoter and precore region in patients who were infected with HBV genotype A

Table 2 compares the frequency of the wild-type in the core promoter and precore region among patients infected with HBV/A who presented with various liver diseases. The wild-type precore region with G1896 was detected in 61 of the 66 (92%) patients for whom the analysis was possible. All the 14 asymptomatic carriers were negative for HBeAg in serum, and the wild-type core promoter with A1762/G1764 was detected in only 43% of them. The wild-type core promoter was found in all 20 patients with acute hepatitis who had serum HBeAg at presentation and in whom HBV infection was resolved. Of the 5 patients with acute hepatitis who went on to chronicity, all of whom had serum HBeAg, 4 (80%) were infected with HBV/A of the wild-type in the core promoter, a frequency comparable to that in 10 of the 14 (71%) patients with HBeAg-positive chronic hepatitis. Patients with HBeAg-negative chronic hepatitis possessed the wild-type core promoter (38%) and precore region (88%) somewhat less frequently than the others.

The 1858th nt of T or C in patients infected with HBV/A at the time of presentation is shown in Table 3. T1858 was not detected in any patients with acute hepatitis in whom HBV infection was resolved, but it was found in one of the 5 (20%) patients who acquired persistent infection, as well as in 24%–32% of those with the other categories of liver disease. T1858 was

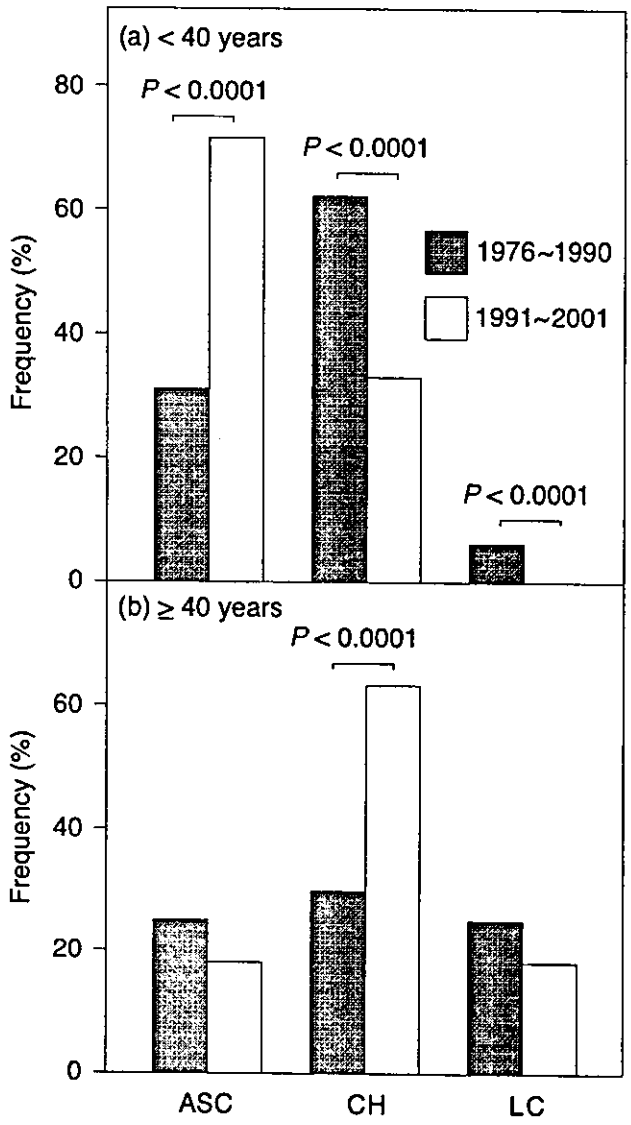

Fig. 2a,b. Comparison of the proportions of patients infected with HBV genotype A aged less than 40 years (**a**) and aged 40 years or older (**b**) who visited Toranomon Hospital between 1976 and 1990 and between 1991 and 2001

detected significantly more frequently in asymptomatic carriers than in patients with acute hepatitis (36% vs 4%; $P = 0.002$), and the difference was even more prominent between asymptomatic carriers and patients with acute hepatitis in whom HBV infection was resolved (36% vs 0%; $P = 0.008$).

Subtypes A and A′ of genotype A

HBV DNA sequences of the large S gene were determined in nucleic acids extracted from 68 patients infected with HBV/A, and subtypes A and A′ were analyzed phylogenetically (Table 4). Subtype A was detected in 53 (80%) patients, and subtype A′ in 4 (6%);

Table 2. Wild-types of core promoter and precore sequence in HBV DNA from patients infected with HBV genotype A at presentation

Liver disease	Wild-type core promoter (A1762/G1764)	Wild-type precore region (G1896)
ASC		
HBeAg (−) (n = 14)	6 (43%)	13 (93%)
AH: Resolved		
HBeAg (+) (n = 20)	20 (100%)	20 (100%)
HBeAg (−) (n = 1)	ND	ND
AH: Persisted for >6 months		
HBeAg (+) (n = 5)	4 (80%)	5 (100%)
CH		
HBeAg (+) (n = 14)	10 (71%)	14 (100%)
HBeAg (−) (n = 8)	3 (38%)	7 (88%)
LC		
HBeAg (−) (n = 2)	1 (50%)	2 (100%)

ND, not detectable

Table 3. Nucleotide 1858 in HBV DNA samples from patients who were infected with HBV genotype A and presented with various liver diseases

Nucleotide at position 1858	ASC (n = 14)	AH: Resolved (n = 21)	AH: Persisted (n = 5)	CH (n = 22)	LC (n = 4)	Total (n = 66)
	P = 0.008					
T	5 (36%)	0	1 (20%)	7 (32%)	1 (25%)	14 (21%)
C	6 (43%)	18 (86%)	4 (80%)	15 (68%)	1 (25%)	44 (67%)
Deletion	3 (21%)	0	0	0	0	3 (5%)
Not detectable	0	3 (14%)	0	0	2 (50%)	5 (8%)

Table 4. Subtypes in patients infected with HBV genotype A

Subtypes	ASC (n = 14)	AH: Resolved (n = 21)	AH: Persisted (n = 5)	CH (n = 22)	LC (n = 4)	Total (n = 66)
A	11 (79%)	18 (86%)	4 (80%)	18 (82%)	2 (50%)	53 (80%)
A′	1 (7%)	0	0	3 (14%)	0	4 (6%)
Untypeable	2 (14%)	3 (14%)	1 (20%)	1 (4%)	2 (50%)	9 (14%)

distinction between A and A′ was not possible in the remaining 9 (14%) patients. Of the 57 patients in whom subtypes were determined, A was detected in 53 (80%) and A′ in the remaining 4 (6%). The 53 patients infected with subtype A, for whom subtyping was feasible, included 11 of the 13 (85%) asymptomatic carriers, 22 of the 22 (100%) with acute hepatitis, 18 of the 21 (85%) with chronic hepatitis, and both patients with liver cirrhosis. Of the 4 patients infected with subtype A′, 1 presented with the asymptomatic carrier state and the remaining 3 with chronic hepatitis.

Table 5 lists the demographic, histopathological, and virological features of the four patients infected with subtype A′. They comprised two males and two females, and two of them were in their twenties and the remaining two in their forties. Two patient with chronic hepatitis and infected with subtype A′ (patient 3 and 4) cleared HBsAg from serum during about 3 years of follow up.

Table 5. Characteristics of the four patients who were infected with HBV of subtype A'

Features	Patient 1	Patient 2	Patient 3	Patient 4
Sex	M	F	F	M
Age (years)	26	27	43	47
Family history		Father Brother		
Diagnosis	ASC	CH	CH	CH
Liver histology	A0/F0	A1/F1	A1/F1	A1/F2
HBV DNA (LGE/ml)	<3.7	≥8.7	<3.7	≥8.7
HBeAg	±	±→±	±	±→±
Nucleotide 1858	C	C	T	C
Wild-type				
Core promoter	No	Yes	Yes	No
Precore sequence	Yes	Yes	Yes	Yes

A, grade of necroinflammatory activity; F, stage of fibrosis; LGE, log genome equivalent

Discussion

In Japan, by far the greatest number of carriers contracted their HBV infection perinatally through mother-to-bady transmission decades ago.[15,16] By contrast, individuals in Europe and the United States acquire HBV infection in adulthood. There are remarkable differences in the distribution of HBV genotypes between Japan and western countries. Genotypes B and C are prevalent in Japan, in contrast to genotypes A and D common in European countries,[4,5] while all seven HBV genotypes are found in the United States, with the distribution dependent on the ethnicity of carriers.[17]

We previously reported that the proportion of patients with acute hepatitis infected with HBV/A in the Tokyo metropolitan area was significantly higher ($P < 0.0001$) than that in those with chronic hepatitis who visited the same hospital.[18] In the present study, a mother with HBV infection was not reported by any of the patients infected with HBV/A who presented with acute hepatitis. Only one patient infected with HBV/A, with chronic hepatitis, was found to have a mother with HBV infection. Her genotype was B, however, thereby excluding mother-to-baby transmission in this patient. These results indicate that, at least in Tokyo, most infection with HBV/A occurs horizontally rather than perinatally, in corroboration with our previous findings.[18]

It may be surprising that 5 of the 26 (19%) patients with acute hepatitis who were infected with HBV/A failed to clear infection. They had all contracted de-novo HBV infection, because they did not test positive for HBsAg in health check-ups before they came down with acute hepatitis. In Japan, acute HBV infection in adulthood evolves into chronicity in only fewer than 1%, unlike that in western countries, where it persists in approximately 10%.[19] It appears that primary infection with HBV of genotype A would tend to be chronic more frequently than those of the other genotypes, even when it is contracted in adulthood.

There is evidence in support of a role of homosexuality in HBV/A infection. A cluster of HBV/A strains was identified in men having sex with men in Amsterdam.[20] In the present study, the most common route of infection with HBV/A in patients with acute hepatitis was homosexual activity, as reported by 11 of 28 patients (39%). HBV/A infection persisted in 4 of the 11 (36%) who had contracted infection by homosexual contacts. There are increasing numbers of young male homosexuals in the Tokyo metropolitan area, some of whom may be at increased risk of infection with HBV/A as well as human immunodeficiency virus type 1.[21] It is worrying that the persistence of acute HBV/A infection will increase in Japan in the future. For preventing the chronicity of HBV/A infection, hepatitis B vaccine would be effective in persons at risk of contracting infection by sexual contacts, in particular.

HBV/A isolates have the nucleotide 1858 of C (C1858) that makes a pair with T1896. Therefore, the G-to-A point mutation in the precore region creating the stop codon is prevented in HBV/A isolates, because A1896 is incompatible with C1858 and destroys the conformation of the pregenome encapsidation signal.[9,10] Hence, the precore mutation (A1896) in HBV/A isolates always accompanies C-to-T mutation at nt 1858. T1858 in HBV/A infection was detected in 5 of the 14 (36%) asymptomatic carriers (all of whom were without serum HBeAg), this being significantly more frequent than in none of the 21 patients in whom acute hepatitis had resolved ($P = 0.008$); 20 of them (95%) presented with HBeAg in serum.

HBV/A isolates in South Africa are phylogenetically different from those in Europe and the United States, and most of them are of subtype A'.[7,22] Subtyping of HBV/A isolates into A (the original genotype A) and

A′ was feasible in 58 (85%) of the 68 patients in the present study. The subtype was A in 53 of these 57 (93%) HBV/A isolates, and A′ in the remaining 4. Where the HBV/A strains of subtype A′ came from, and how they have spread in Tokyo, is not certain, and these questions need to be sorted out in future epidemiological studies. All HBV/A infections of subtype A′ occurred in persistent HBV infection and occurred in 1 asymptomatic carrier and 3 patients with chronic hepatitis, of whom 1 cleared infection during 3 years of follow-up.

How these four patients contracted HBV infection of subtype A′ is a matter of conjecture. Although they all had chronic HBV infection, a possibility remains that there had been evolution of acute infection with subtype A′, which may have been acquired through sexual contacts. Special care will have to be devoted to them, because infection with subtype A′ in Africa is associated with a high incidence of hepatocellular carcinoma,[23] although its development would be accelerated by afratoxin there.

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Interferon monotherapy for patients with chronic hepatitis C and normal serum aminotransferase levels at commencement of treatment

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Background. Approximately 30% of patients with chronic hepatitis C have normal serum alanine amino transferase (ALT) levels. While interferon (IFN) monotherapy is approved for patients with chronic hepatitis C infection, the effectiveness of such therapy for chronic hepatitis C patients with normal ALT levels at commencement of treatment remains poorly understood. ***Methods.*** Ninety-four individuals (M/F, 54:40; median age, 46 years) with normal ALT levels (< 50 IU/l) at the commencement of treatment who were positive for both anti-hepatitis C virus (HCV) and serum HCV-RNA were studied. Among this group, 18 individuals (M/F, 9:9; median age, 50 years) had had persistently normal ALT levels for at least 3 months prior to treatment. All patients received their first course of IFN therapy in this study. ***Results.*** Forty-three (45.7%) of 94 individuals had lost serum HCV-RNA at 6 months after cessation of therapy (complete response; CR). The proportion of patients with genotype 2a and HCV-RNA level over 1 Meq/ml who showed CR was significantly lower in those with normal ALT levels than in those with elevated ALT levels (23.8% vs 55.6%; $P = 0.0189$). Two patients who had persistently normal ALT levels and HCV-RNA level over 1 Meq/ml were non-responders (NR) and had ALT flare-ups after IFN therapy. Patients with HCV-RNA levels of less than 1 Meq/ml did not show differential responses based on ALT levels. ***Conclusions.*** Our data suggest that IFN therapy is effective for patients with normal ALT levels and less than 1 Meq/ml HCV-RNA. Thus, such patients should be considered for curative IFN therapy.

Key words: interferon, chronic hepatitis C, normal ALT

Introduction

Antiviral treatment for patients with chronic hepatitis C virus (HCV) infection has generally been limited to those with significantly abnormal serum transaminase activity. Results of interferon (IFN) monotherapy in patients with normal serum alanine amino transferase (ALT) levels were the basis for the conclusions of the 1997 Consensus Conference that these patients should not be treated.[1] Thirty of 52 patients treated in seven studies, for whom data were available, had de novo elevations of ALT levels during therapy, and some patients continued to have ALT elevation after IFN was discontinued.[2–8] Approximately 30% of patients with chronic hepatitis C have normal ALT levels.[9] It is true that if ALT levels are persistently normal, the possibility of significant and progressive liver disease tends to be low, but a significant proportion of patients with persistently normal ALT levels show some histological signs of fibrosis—the degree of which is usually mild but is sometimes more marked—and in rare cases, cirrhosis may be present.[1] IFN treatment is effective for patients with chronic hepatitis C, reducing ALT levels, improving histological activity,[10,11] and eliminating HCV-RNA.[12–15] However, the effectiveness of IFN therapy for patients with normal ALT remains poorly understood. Few studies have compared patients with elevated ALT levels and matched patients with normal ALT levels with respect to assessing sustained virological response rates.[1] Moreover, the changes in ALT levels after IFN therapy in patients with normal ALT levels before treatment is not clear.

The aim of this retrospective study was to determine the incidence of sustained virological response after IFN therapy and the changes in ALT levels after therapy in patients with normal ALT levels at commencement of therapy.

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Patients and methods

Patients

Between July 1992 and August 2001, all anti-HCV-positive patients treated at Toranomon Hospital, Tokyo, Japan, were recruited for the present study, and assessed by routine work-up. Routine evaluation of patients included medical history, physical examination, complete blood count, and measurement of ALT and markers for viral hepatitis. Patients coinfected with hepatitis B virus (HBV); those with autoimmune diseases; those with previous IFN treatment or medication for hepatitis; history of heavy alcohol abuse; liver cirrhosis or hepatocellular carcinoma on ultrasonography; or coexisting cardiac, renal, pulmonary endocrine conditions or psychiatric conditions, were excluded from this study. We retrospectively identified 791 patients who were first treated with IFN (Table 1). Normal ALT level was defined at ALT less than 50 IU/l at the commencement of IFN therapy. Of the 791 patients, 94 had normal ALT levels (M/F, 54:40; median age, 46 years) at the commencement of IFN therapy. Among this group, 18 patients (M/F, 9:9; median age, 50 years) had had persistently normal ALT levels for a mean period of 8 months (range, 3–91 months) before IFN therapy. In all patients, ALT level was examined every month before treatment, during treatment, and during the subsequent 6-month follow-up period.

Histopathological examination of liver biopsy specimens

Patients underwent liver biopsy within 6 months prior to the commencement of IFN therapy. The baseline liver histology of chronic hepatitis was classified into four stages according to the extent of fibrosis: stage 0 (F0), no fibrosis; stage 1 (F1), periportal expansion; stage 2 (F2), portoportal septa; and stage 3 (F3), portocentral linkage or bridging fibrosis.[16] No patients with liver cirrhosis (F4) were included in this study.

Serum HCV-RNA marker

Qualitative analysis of HCV-RNA was performed using a branched DNA probe assay (bDNA probe assay, version 2.0; Chiron, Dai-ichi Kagaku, Tokyo, Japan). HCV genotype was classified by polymerase chain reaction (PCR), using a mixture of primers for six subtypes known to exist in Japan, as reported previously.[17]

Interferon (IFN) treatment and definition of response to IFN therapy

The IFN treatment consisted of 6 to 10 million units (MU) of natural IFN alpha or natural IFN beta. IFN treatment was administered daily for 2 months or daily for 2 months and then three times a week for 4–5 months. After discontinuation of the therapy, all patients were followed-up for at least an additional 6-month period. A complete response (CR) was defined as negative HCV-RNA by PCR at two time points, 3 and 6 months after the completion of IFN therapy. Incomplete response (IR) was defined as normalization of serum ALT, but positive HCV-RNA by PCR at two time points, 3 and 6 months after cessation of IFN therapy. All patients other than those with CR and IR were considered nonresponders (NR).

Table 1. Comparison of patients with chronic hepatitis C virus (HCV) infection who had elevated or normal serum alanine aminotransferase (ALT) levels

	Elevated ALT (n = 697)	Normal ALT (n = 94)	P
Age (years)[a]	51 (17–73)	46 (20–68)	0.022
Sex (male/female)	436/261	54/40	0.40
Liver histology (F0/F1/F2/F3/N)[b]	3/446/180/48/20	1/82/5/1/5	<0.0001
HCV genotype (1b/2a/2b)	466/176/55	38/43/13	<0.0001
HCV-RNA (≤1 Meq/>1 Meq) (Meq/ml, bDNA probe, version II)	235/459	48/46	0.0016
Serum ALT (IU/l)[a]	102 (51–683)	36 (11–50)	<0.001
Alpha/Beta IFN (alpha %)	541/156 (77.6)	69/25 (73.4)	0.95
Total dose of IFN (MU)[a]	624 (261–1040)	627 (318–1040)	0.924
Duration of IFN therapy (weeks)[a,c]	24 (8–30)	24 (8–28)	0.483

[a] Data values are expressed as medians with ranges in parentheses unless indicated otherwise
[b] Liver fibrosis assessed on a five-point scale: F0, no fibrosis; F1, periportal expansion; F2, portoportal septa; F3, portocentral linkage or bridging fibrosis; F4, cirrhosis; N, liver biopsy not performed
[c] IFN therapy was administered daily for 2 months or daily for 2 months and three times/week for 4 months

Statistical analysis and ethical considerations

Differences between groups were examined for statistical significance, using the Mann-Whitney test (*U*-test) and the χ^2 test where appropriate. A two-tailed *P* value of less than 0.05 was considered statistically significant. Independent predictive factors associated with CR to IFN treatment were studied using stepwise Cox regression analysis. Potential predictive factors for CR to IFN treatment that were assessed included the following nine variables: age (≤51 vs >51 years), sex, severity of liver disease (F0 and F1 vs F2 and F3), ALT (≤50 vs >50 IU/l), type of IFN (alpha vs beta), period of IFN therapy (≤24 vs >24 weeks), total dose of IFN (≤624 vs >624 MU), HCV genotype (1 vs other than 1), and HCV RNA level (≤1 vs >1 Meq/ml). All factors found to be at least marginally associated with CR to IFN treatment ($P < 0.15$) were entered into a multivariate multiple logistic regression. The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative-risk confidence. All analyses described above were performed using the SPSS program (version 7.5, SPSS, Chicago, IL, USA).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Results

Clinical and virological features of patients with normal ALT

Table 1 lists the demographic and clinical characteristics of patients with elevated ALT levels and those with normal ALT levels. There were 94 patients with normal ALT levels (group N), with a median age of 46 years (range, 20–68 years). Liver biopsy was performed in all but 5 patients, and 82 patients (87.3%) were assessed as F1 stage. Liver histology in group N tended to be milder compared with that in group E (patients with elevated ALT). HCV genotype 2a tended to be more prevalent in group N than in Group E. Among the patients in group N, 18 had persistently normal ALT levels (median age, 50 years), and were assessed as showing histological stage F1.

Virological response to IFN therapy

The overall CR rate in patients in group N was 45.7% (43/94 patients). Table 2 shows the virological response to IFN therapy in patients with chronic HCV infection for group E and group N. Each HCV genotype and HCV-RNA level were compared between the two groups. No patients in group N with genotype 1b and HCV-RNA level over 1 Meq/ml showed a CR to IFN therapy. Among patients with genotype 2a and HCV-RNA levels more than 1 Meq/ml, the CR rate was significantly lower in group N than in group E (23.8% vs 55.6%; $P = 0.0189$). However, there was no difference between group N and group E among patients of all genotypes with HCV-RNA levels 1 Meq/ml or less. Moreover, in patients with HCV-RNA level over 1 Meq/ml, there were no differences in CR rate and the severity of liver fibrosis between the two groups. After IFN therapy, 26.6% (25/94) of patients in group N had HCV-RNA levels that returned to pretreatment values and had ALT flare-ups during follow-up.

The virological response to IFN therapy in the 18 patients with chronic HCV infection and persistently normal ALT levels is shown in Table 3. The overall CR rate was 38.9% (7/18 patients). Evaluation according to genotype was difficult, as there were few patients with genotype 2b. Among patients with HCV-RNA levels of 1 Meq/ml or less with genotypes 1b and 2a, the effects of IFN therapy were the same as for those in group E. However, the rate of CR to IFN therapy in patients with HCV-RNA levels of more than 1 Meq/ml tended to be low.

Table 2. Proportion of patients with chronic hepatitis C virus infection who showed complete response (CR) to interferon (IFN) therapy, according to HCV genotype and HCV-RNA level

	Elevated ALT group (n = 697)	Normal ALT group (n = 94)	P
Genotype 1b			
≤1 Meq/ml	57/125 (45.6)	15/22 (68.1)	0.085
>1 Meq/ml	36/341 (10.6)	0/16 (0)	0.3442
Genotype 2a			
≤1 Meq/ml	74/95 (77.9)	17/22 (77.3)	1.0
>1 Meq/ml	45/81 (55.6)	5/21 (23.8)	0.0189
Genotype 2b			
≤1 Meq/ml	12/15 (80)	3/4 (75)	1.0
>1 Meq/ml	9/37 (24.3)	3/9 (33.3)	0.897

Complete response (CR): sustained loss of serum HCV RNA was observed for 3 and 6 months after treatment
Data values in parentheses show percentages of patients

Table 3. Proportion of patients with chronic hepatitis C virus infection and persistently normal serum alanine aminotransferase (ALT) levels who showed complete response (CR) to interferon (IFN) therapy, stratified according to HCV genotype and HCV-RNA level

	HCV RNA	
HCV genotype	≤1 Meq/ml	>1 Meq/ml
1b	2/3 (66.6%)	0/3 (0%)
2a	2/3 (66.6%)	2/8 (25%)
2b	No participant	1/1 (100%)

Data values in parentheses show percentages of patients

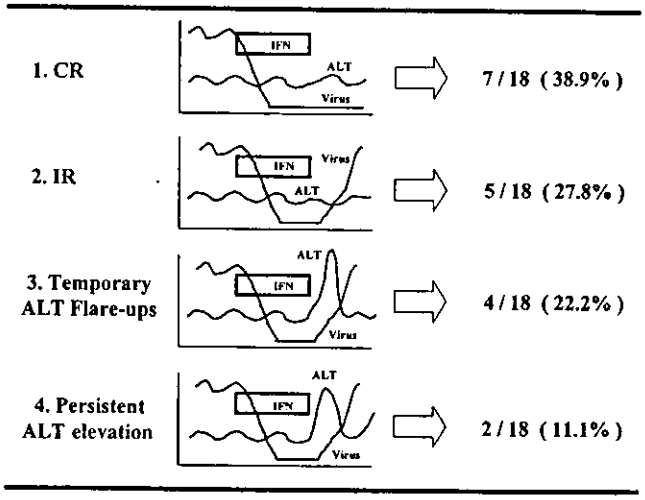

Fig. 1. Virological response and serum alanine aminotransferase (*ALT*) changes during and after interferon (*IFN*) therapy in 18 patients with hepatitis C virus (HCV) infection and persistently normal ALT levels before treatment. *CR*, complete response; *IR*, incomplete response. Data *on the right* show the number of patients showing the particular response/total number of patients in that group, with percentages of patients shown in *parentheses*

Changes in ALT levels after IFN therapy in patients with persistently normal ALT levels

Eighteen patients with persistently normal ALT levels were followed-up after IFN therapy, for a mean duration of 39 months (range, 11–65 months). Figure 1 shows the virological response and ALT changes during and after IFN therapy. Five patients had an IR (27.8%), and four had temporary ALT flare-ups after IFN therapy (22.2%). The five patients with IR had two types of genotype; three patients were 1b and two were 2a. Four patients with IR had an HCV-RNA level over 1 Meq/ml at the commencement of IFN therapy, and their HCV RNA became undetectable during IFN therapy. In the remaining patient, HCV RNA was detectable during therapy. The ALT levels of these five patients were normalized during and after therapy. The four patients with temporary ALT flare-ups had two types of genotype; one was 1b and three were 2a. Three patients with temporary ALT flare-ups had an HCV-RNA level over 1 Meq/ml at the commencement of IFN therapy, and in two of these, HCV RNA levels became undetectable during therapy. However, HCV RNA in another patient was detectable, while it was not examined during therapy in the remaining patient. ALT levels in these patients were normalized during and after therapy. These temporary ALT flare-ups occurred at a mean time point of 3 months posttreatment (range, 2–4 months). Finally, two patients showed persistent ALT elevation after IFN therapy (11.1%); one patient was genotype 1b while the other was 2a. HCV RNA levels in one patient (genotype 2a) were 2.7 Meq/ml at 3 months pretreatment and had increased to 9.2 Meq/ml at the commencement of treatment. HCV RNA in this patient was undetectable during therapy. The HCV RNA level in the other patient (genotype 1b) was 36 Meq/ml at the commencement of treatment and was always detectable during therapy.

Multivariate analysis of predictive factors for response to IFN treatment

We explored the predictive factors for response to IFN therapy in patients with normal ALT levels. Among the nine factors examined in the univariate analysis, only HCV RNA level significantly influenced the response to IFN therapy ($P < 0.0001$). In comparison, HCV genotype showed borderline significance, with a higher chance of sustained response ($P = 0.072$). As these two variables were mutually correlated, multivariate analysis was performed. In the last step, the following variables were entered into the model and could not be removed: HCV RNA level ($P < 0.0001$) and HCV genotype ($P = 0.022$; Table 4).

Finally, we explored the predictive factors for response to IFN therapy in all patients in both groups. On univariate analysis, the following six factors significantly influenced the response to IFN therapy: HCV RNA level ($P < 0.0001$), HCV genotype ($P < 0.0001$), total IFN dose ($P < 0.0001$), severity of liver disease ($P = 0.0005$), ALT ($P = 0.0087$), and age ($P = 0.0285$). As the variables were mutually correlated, multivariate analysis was performed. In the last step, the following variables were entered into the model and could not be removed: HCV RNA level ($P < 0.0001$), HCV genotype ($P < 0.0001$), age ($P = 0.0023$) and total IFN dose ($P = 0.0084$; Table 4).

Discussion

The clinical effectiveness of IFN monotherapy for chronic hepatitis C associated with normal serum ALT levels has been reported in numerous studies.[1-8] At the 1997 National Institutes of Health Consensus Development Conference on "Management of Hepatitis C," the Consensus Panel concluded that alpha IFN treatment could not be recommended for patients with persistently normal ALT levels and that the therapy might actually worsen the course of the disease.[18] The basis of this conclusion regarding IFN monotherapy in patients with normal ALT levels was that 30 of 52 treated patients in seven studies, for whom data were available, had had de novo elevations of ALT levels during therapy, and some patients continued to exhibit ALT

Table 4. Independent variables contributing to complete response to IFN therapy, on multivariate analysis

Variable	Multivariate odds ratio	95% Confidence interval[a]	*P* Value
Multivariate analysis of patients with normal ALT levels			
HCV RNA level (≤1 vs >1 Meq/ml)	0.047	0.015–0.15	<0.0001
Genotype (1 vs other than 1)	3.81	1.21–11.99	0.022
Multivariate analysis of all patients (both normal and elevated ALT levels)			
HCV RNA level (≤1 vs >1 Meq/ml)	0.17	0.12–0.25	<0.0001
Genotype (1 vs other than 1)	4.49	3.13–6.46	<0.0001
Age (≤51 vs >51 years)	0.57	0.39–0.82	0.0023
Total dose of IFN (≤624 vs >624 MU)	1.62	1.13–2.32	0.0084

[a] Values are the odds of having a sustained response to interferon

elevations after discontinuation of IFN.[2–8] In the seven studies, the overall CR rate was only 19%. However, in comparison, the CR rate for patients with elevated ALT levels receiving IFN monotherapy for 24 weeks was only 12%. Although the CR rate for these patients with normal ALT levels was comparable to or even higher than that for patients with elevated ALT levels, few studies had matched these two groups for other important variables, such as HCV genotype, patient age, or underlying disease severity in assessing CR rates.[1] To elucidate the efficacy of IFN therapy for these patients, considering HCV genotype and HCV-RNA level, we evaluated 94 consecutive patients with normal ALT levels at commencement of therapy in our study. We found that no patient with normal ALT levels, genotype 1b, and HCV-RNA levels over 1 Meq/ml showed a CR to IFN therapy. Moreover, among patients with genotype 2a and HCV-RNA levels over 1 Meq/ml, the CR rate was significantly lower in patients with normal ALT levels than in those with elevated ALT levels.

Why did patients, especially those with genotype 2a, with normal ALT levels and HCV-RNA levels over 1 Meq/ml, have a poorer response to IFN monotherapy? Although the effect of IFN therapy is influenced by HCV-RNA levels and/or genotypes, other factors, such as the host immune response to IFN, may influence the overall response. In patients with normal ALT levels, there may be an equilibrium between HCV replication and the host immune response.[18] Under this condition, the efficacy of IFN may decrease. However, the exact reason for this finding is not clear at present, and further studies are required at a genetic level. On the other hand, when HCV-RNA levels were less than 1 Meq/ml, there was no difference in the CR rate of patients between those with normal ALT levels at commencement of treatment and those with elevated ALT levels, independent of HCV genotype. Ohmiya et al.[19] also reported that the HCV-RNA level was associated with CR to natural IFN alpha in patients with normal ALT levels. Moreover, our univariate and multivariate analyses revealed that HCV RNA level was the most important factor in the response to IFN therapy among patients with normal ALT levels. Therefore, a low HCV-RNA level may be a favorable marker for CR in patients with normal ALT. On the other hand, univariate analysis showed that an elevated ALT level (>50 IU); odds ratio [OR], 1.76 (95% confidence interval [CI], 1.14–2.74); $P = 0.011$] was associated with a high probability of CR. However, multivariate analysis showed that ALT level did not independently or significantly influence the outcome of IFN therapy (Table 4; ALT not shown, because of lack of significance). Other factors, such as HCV RNA level and genotype, were more important.

Current evidence suggests that the treatment of patients with persistently normal ALT levels is sometimes not beneficial, and may actually induce liver enzyme abnormalities,[1] which may persist after completion of IFN treatment. The percentage of patients with ALT flare-ups after IFN therapy was reported to be 40% to 70% among patients with normal ALT levels before treatment.[2,20–22] In our study, 6 of 18 (33.3%) IFN-treated patients with persistently normal ALT levels before treatment showed ALT flare-ups posttreatment. Although virological relapse accompanied by increased ALT activity has been reported previously, the enzyme levels returned to normal values within 6 months.[20] It was speculated that these cases might reflect IFN-induced activation of a cytolytic response against infected hepatocytes.[23] In line with this hypothesis, we found that ALT activity returned to normal values after IFN withdrawal. In our study, when temporary ALT flare-ups appeared in patients who had had persistently normal ALT levels, the duration of the rise was, on average, 3 months (range, 2–4 months). However, in two patients, the ALT level remained elevated after more than 1 year (12 and 15 months, respectively) after the therapy had been finished. HCV RNA levels in these patients were high. We speculate that the IFN therapy may have destroyed the equilibrium between

HCV replication and the host immune response in these two patients. In these two patients, persistently active hepatitis may have been induced by the IFN therapy.

Since the advent of combination therapy using IFN and ribavirin, and, more recently, combination therapy using pegylated IFN (peginterferon) and ribavirin, the CR rates for HCV infection have shown a dramatic improvement.[24,25] In all studies of chronic hepatitis C patients with normal or near-normal ALT levels, the CR rates with the combination therapies have been similar to the rates reported in patients with abnormal ALT levels.[26–30] These more potent treatments may, therefore, be beneficial for patients with normal ALT levels before treatment. However, the side effects of these combination therapies have become a topical issue, and may preclude some patients from receiving combination therapy. For these patients, IFN monotherapy may be necessary, and the timing of the IFN therapy is, therefore, important.

In conclusion, we investigated the effect of IFN monotherapy in 94 patients with chronic hepatitis C infection who had had normal ALT levels at the commencement of therapy, including 18 patients with who had had persistently normal ALT levels. Among patients with genotype 2a and HCV-RNA levels over 1 Meq/ml, the CR rate was significantly lower in those with normal ALT levels than in those with elevated ALT levels. There was no difference in response according to ALT levels among patients with HCV-RNA levels of 1 Meq/ml or less. Our data suggest that IFN therapy for patients with normal ALT levels before treatment and HCV-RNA of 1 Meq/ml or less was effective. Moreover, ALT levels flared up after IFN therapy in some patients who had had persistently normal ALT levels before treatment. Our results indicate that, in patients with HCV-RNA levels of more than 1 Meq/ml, IFN therapy should be commenced only after there is an increase in the ALT level.

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