濃染などがある．黄疸を指摘されて受診する場合も少なくない．多くの場合，発熱も認める．また，心窩部痛や右上腹部痛などを主訴として受診する場合もある．肝臓内にはもともと痛覚はないが，肝被膜には痛覚があり，急性肝炎に伴う肝の腫大によりこの被膜が伸展されてこのような症状が出ると考えられる．なお，潜伏期間は約1カ月である．

臨床検査所見と画像所見

採血検査では血清AST・ALT・LDHの著明な上昇を認める．TTT(チモール混濁試験)・ZTT(硫酸亜鉛混濁試験)も高値となるが，とりわけTTTの高値はA型急性肝炎に特徴的である．また時としてγ-GTP・ALP・T-Bilの上昇が著明で，胆汁うっ滞が前景に立つ場合があり，他の胆汁うっ滞をきたす疾患との鑑別が重要となる．一方，HPT・PT値の低下は急性の肝障害に伴う肝機能の低下を示唆するものである．

A型肝炎の診断は血清学的に血液中にIgM型のHAV抗体を検出することによる．一般的には他の急性肝炎の原因となりうるウイルス感染の血清学的検索と同時に行うこととなる．A型肝炎以外に急性肝障害の原因としてスクリーニングすべきものとして，ウイルス感染としてB型肝炎ウイルス・C型肝炎ウイルス・EBウイルス・サイトメガロウイルスがあり，またわが国において近年人畜共通感染症として問題になっているE型肝炎ウイルスについてもスクリーニングが必要である．さらに，自己免疫性肝炎や原発性胆汁性肝硬変症といった慢性肝疾患もその発症時には急性肝障害の形をとる場合があり，検索が必要である．

一方，びまん性の肝疾患である急性肝炎では，胆嚢壁の肥厚(図1)や脾腫を除いて特徴的な画像所見はない．しかし胆道系疾患や腫瘍などの他疾患との鑑別のために画像検査は必要である．血清学的検査でHAV感染が判明し，A型急性肝炎の診断が確定するまでには数日を要するが，一方，総胆管結石や胆道感染症は一刻も早くドレナージなどの治療を要する場合のある疾患である．これら疾患の除外のためにも腹部超音波をはじめとした画像診断は必要である．とりわけ心窩部痛・右上腹部痛などの症状や発熱を伴う場合には必須であろう．

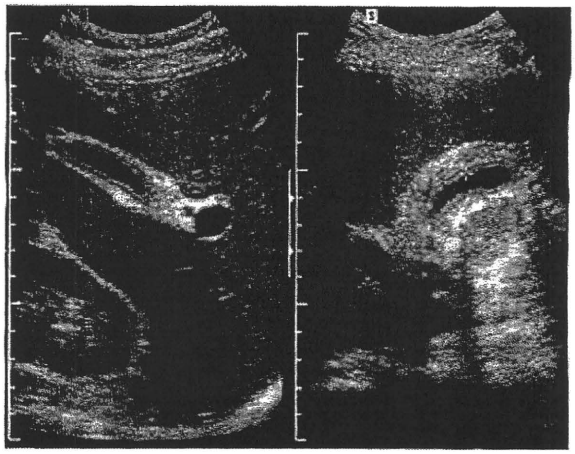

図1　A型急性肝炎の超音波画像

鑑別診断

まず急性肝炎を含めた急性の肝機能異常の鑑別が必要となる．前述のように総胆管結石などを代表とする胆道系疾患や，右心不全によるうっ血肝などを除外する必要がある．次に急性に肝障害を生ずる病態の鑑別が必要である．前述のウイルス感染を含めた各種肝障害の原因の検索に加え，薬剤性肝障害の検索も必要となる．

治療法と臨床経過・予後

入院時にウイルス検索を含めた採血を行い，入院させて安静を保たせつつトランスアミナーゼをはじめとした各種肝機能のデータをfol-

low-up し，原因検索の結果を待つということになる．肝炎に対する投薬は特になく，食事がとれていれば点滴による補液も不要である．一般にトランスアミナーゼの上昇期にはAST＞ALTのことが多く，ピークを過ぎて下降期になるとAST＜ALTとなることが多い．

全身倦怠感や食欲不振のみが症状である時期が最も肝障害が強い(トランスアミナーゼが上昇している)ことが多く，血清総ビリルビン値が上昇して黄疸が出るころには肝障害はピークを越えていることも多い．現実的には全身倦怠感のみがある時期には何となく理由もわからないため我慢して受診せず，尿濃染や黄疸が出てあわてて受診し入院する患者の多くは，入院時にすでに肝障害のピークは過ぎていることも少なくない．

前述のごとくA型肝炎は慢性化することはなく，劇症化することなくピークを過ぎれば肝障害は鎮静化し最終的に肝機能は正常化する．ただし稀にトランスアミナーゼの上昇が二峰性を呈する場合や治癒が遷延する例もあり，注意が必要である[4]．劇症化は約1％であり，劇症肝炎の約8％を占める．このうち死亡例は約20％である[5]．

予防法・ワクチン

A型肝炎流行地における予防法は，他の感染症に共通する手洗い・洗顔であり，熱を通さない飲食物は避けることである．わが国では細胞培養による不活化ワクチンであるA型肝炎ワクチンが予防の主体となっており，海外赴任者，長期旅行者，集団および家族内発生時の周辺の人々，調理・食品関係者，保育関係者，同性愛者，重症肝疾患患者，医療従事者などが対象になると考えられる．

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Prevalence of Low-Level Hepatitis B Viremia in Patients with HBV Surface Antigen-Negative Hepatocellular Carcinoma with and without Hepatitis C Virus Infection in Japan: Analysis by COBAS TaqMan Real-Time PCR

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Key Words
Hepatocellular carcinoma · HBV surface antigen-negative · COBAS TaqMan HBV test · HBV DNA, circulating low-level

Abstract
Objectives: The effect of circulating low-level hepatitis B virus (HBV), defined as one of the states of 'occult HBV infection', on the development of hepatocellular carcinoma (HCC) in HBV surface antigen (HBsAg)-negative patients is controversial. In addition, the prevalence of occult HBV infection strongly depends on the sensitivity of the HBV detection method. We investigated the prevalence of low-level HBV in the serum of HBsAg-negative patients with HCC using a newly developed, sensitive method based on real-time polymerase chain reaction. ***Methods:*** Serum was examined for HBV DNA in 132 patients with HBsAg-negative HCC (95 with hepatitis C virus [HCV] infection and 37 without detectable hepatitis virus infection) with the COBAS TaqMan HBV test, of which the 95% hit rate is 35 copies/ml (6.7 IU/ml). ***Results:*** Low-level HBV DNA was detected in 2 of 95 (2.1%) patients with HCV-related HCC and 1 of 37 (2.7%) patients with non-viral HCC. ***Conclusion:*** The prevalence of the detection of circulating low-level HBV was low in both HBsAg-negative HCC patients with HCV infection and those without detectable hepatitis virus, even with the use of the most sensitive method for the detection of HBV. Circulating low-level HBV does not appear to play an important role in hepatocarcinogenesis in HBsAg-negative HCC.

Introduction

Occult hepatitis B virus (HBV) infection is a state of persistent HBV infection that was identified after the development of sensitive polymerase chain reaction (PCR) assay. In such a state, a low level of HBV DNA is detected in serum samples and/or liver tissue of individuals who are HBV surface antigen (HBsAg)-negative. Occult HBV infection has been documented in a number of patient subgroups including those with hepatitis C virus (HCV) infection, human immunodeficiency virus (HIV) infection, and cryptogenic liver disease, and the clinical implications of occult HBV infection have been reported with respect to disease progression, treatment efficacy, and the development of hepatocellular carcinoma (HCC) in patients with HBV marker-negative chronic liver disease [1–3].

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Several researchers have reported the effect of occult HBV infection on the development of HCC in HBV virus marker-negative patients with HCV infection and in those without hepatitis virus infection, but the results are controversial [1–4]. Some studies investigated the presence or absence of HBV DNA in liver tissue (HCC and/or noncancerous part), whereas other studies investigated this in serum samples. In addition, the detection methods differed and the sensitivities varied, resulting in differences in the prevalence found even within the same patient population. Increasing the sensitivity of HBV detection will contribute to the precise determination of the prevalence of occult HBV infection in patients with HCC.

In the present study, we investigated the prevalence of circulating low-level HBV in HCC patients in whom the HBV surface antigen (HBsAg) was negative by means of the COBAS TaqMan HBV test. This recently developed test is based on real-time PCR and has high sensitivity for the detection of HBV and can detect HBV of 50 copies/ml [5]. We analyzed two subgroups of patients with HCC, patients with HCV infection and those without hepatitis virus infection.

Table 1. Patient background

	HCV-related HCC (n = 95)	Non-viral HCC (n = 37)
Age, years	65.9 ± 6.7	68.1 ± 7.9
Sex, female/male, n	36/59	14/23
Anti-HBs (+/–), n	27/68	9/28
Anti-HBc (+/–), n	49/46	19/18
Cirrhosis, present/absent, n	77/18	29/8
Total bilirubin, mg/dl	0.88 ± 0.57	1.01 ± 0.64
Albumin, g/dl	3.51 ± 0.53	3.48 ± 0.58
15-min retention of ICG, %	21.3 ± 11.9	27.4 ± 15.1
Prothrombin time, %	85.5 ± 16.4	78.6 ± 19.9
Platelet counts, × 10,000/μl	10.6 ± 5.3	12.8 ± 6.7
Child-Pugh class, A/B/C, n	59/32/4	22/13/2
Tumor size, cm	1.80 ± 0.66	2.62 ± 2.30
Number of tumors	1.36 ± 1.21	1.89 ± 1.29
α-Fetoprotein, ng/ml[a]	20.0 (2.5–2,987)	8.0 (1.0–261)
Tumor stage, I/II/III, n	54/33/8	14/13/10

Unless otherwise indicated the values are give as the mean ± SD. Anti-HBs = Hepatitis B virus surface antibody; Anti-HBc = hepatitis B virus core antibody; ICG = Indocyanine-green test.

[a] Median (range).

Patients and Methods

HCC was diagnosed in a total of 1,083 patients at Ogaki Municipal Hospital between 1992 and 2004. HBsAg was positive in 182 of 1,083 (16.8%) patients and it was negative in the remaining 901 patients (83.2%, HBsAg-negative HCC). Stored serum samples for the measurement of HBV DNA was obtained from 132 patients with HBsAg-negative HCC and these samples were tested. Diagnosis was on the basis of histologic examination of tumor tissue taken from resected or biopsy specimens or on the basis of typical imaging findings including a mosaic pattern with a halo on B-mode ultrasonographic images, hypervascularity on angiographic images, and a high-density mass on arterial phase dynamic computed tomography (CT) images with a low-density mass on portal phase dynamic CT images obtained with a helical or multidetector row CT scanner. For all patients, the serum samples that were analyzed in the study were obtained at the time of diagnosis of HCC and stored at –80°C until analysis.

Patients comprised 82 men and 50 women aged 66.5 ± 7.1 years. Serum markers for persistent HBV infection, including HBsAg (measured with ARCHITECT HBsAg QT, Abbott Japan, Tokyo, Japan) and HBV DNA (measured with the Amplicor HBV test, Roche Diagnostics, Branchburg, NJ), were negative in all 132 patients. HCV infection was confirmed in 95 of the 132 patients by detection of both HCV antibody and HCV RNA with the Amplicor HCV test, version 2.0 (Roche Diagnostics) in serum (HCV-related HCC). HCV antibody and HCV RNA were negative in the remaining 37 patients (non-viral HCC). Patient characteristics of both subgroups are shown in table 1. HBV surface antibody (anti-HBs), measured with ARCHITECT anti-HBs (Abbott Japan), was positive in 27 (28.4%) patients with HCV-related HCC and in 5 (13.5%) patients with non-viral HCC. HBV core antibody (anti-HBc), measured with ARCHITECT anti-HBc (Abbott Japan), was positive in 49 (51.6%) patients with HCV-related HCC and in 19 (51.4%) patients with non-viral HCC.

The entire protocol was approved by the hospital ethics committee and carried out in compliance with the Helsinki Declaration. Written informed consent was obtained prior to sampling of stored serum from all patients and prior to the measurement of serum HBV DNA from patients who were alive at the time of the study.

Detection and Quantification of Low-Level HBV DNA in Serum

Detection and quantification of HBV DNA was carried out with the COBAS TaqMan HBV test (Roche Diagnostics) according to Weiss et al. [6]. HBV DNA was manually isolated from a 500-μl of serum sample using a generic preparation sample kit (High Pure 16 System Viral Nucleic Acid Kit, Roche Diagnostics). A known number of quantitation standard (QS) molecules was introduced into each specimen during sample lysis and carried throughout the specimen preparation, amplification, and detection steps, serving as a QS and inhibition control. The DNA was eluted in a volume of approximately 80 μl, of which 50 μl was used for PCR in a reaction mixture of 100 μl, amplifying a 105-bp segment of the precore-core region. The cycles in which the fluorescence becomes detectable for target HBV and QS are used to calculate the target HBV concentration.

Table 2. Patients in whom low-level HBV DNA was detected from serum

Sex	Age years	Child-Pugh class	HCV RNA	Anti-HBs	Anti-HBc	Tumor size, cm	Observation period, days	Outcome	Serum HBV DNA concentration copies/ml
M	75	A	p	p	p	2.9	1,232	alive	60
M	61	A	p	n	n	1.1	2,878	alive	126
F	78	B	n	n	n	2.0	339	dead	98

HCV RNA = Hepatitis C virus RNA; Anti-HBs = hepatitis B virus surface antibody; Anti-HBc = hepatitis B virus core antibody; p = positive; n = negative.

Results and Discussion

HBV DNA was detected in 2 of the 95 (2.1%) HCC patients with chronic HCV infection and in 1 of the 37 (2.7%) HCC patients without hepatitis virus infection. HCC patients in whom low-level HBV DNA was detected by the COBAS TaqMan HBV test are shown in table 2. Anti-HBs and anti-HBc were positive in 1 patient, whereas both were negative in the other 2 patients. All 3 patients in whom HBV DNA was detected had cirrhosis at the time of diagnosis of HCC. The serum HBV DNA level was 60–126 copies/ml.

The association of overt HBV infection with HCC has been established; however, whether the pathogenesis of HCC can be attributed to occult HBV infection remains controversial. The reported frequency of the detection of low-level HBV DNA in serum has varied significantly among HBsAg-negative patients with HCC [7–15].

The calculated prevalence of occult HBV infection depends strongly on the sensitivity of the detection method for HBV DNA. Although HBV DNA PCR is the most sensitive test currently available for detection of HBV DNA molecules, its sensitivity varies. Low sensitivity can result in underestimation of the rate of occult HBV infection and, conversely, a false-positive HBV DNA detection can result in overestimation. A highly sensitive but specific HBV PCR assay, therefore, is essential for determining precisely the prevalence of occult HBV infection.

This is the first study to evaluate the prevalence of low-level HBV DNA in serum by the commercially available COBAS TaqMan HBV test in HBsAg-negative patients with HCC in Japan where the prevalence of HBsAg-positive individuals is reportedly under 1% and where around 15% of HCC develops in HBsAg-positive patients [16]. The COBAS TaqMan real-time HBV test is reported to have high reproducibility and a high range of detection and quantification, in comparison to the COBAS Amplicor HBV test; it can reportedly detect HBV of less than 50 copies/ml; the 95% hit rate is reportedly 35 copies/ml (6.7 IU/ml) [5, 17]. Although the presence of the nick in minus strand of HBV could impair the sensitivity, it still has high sensitivity for the detection of HBV DNA, in comparison to other methods that are commercially available. The HBV virus level in serum is usually less than 10^4 copies/ml in patients with occult HBV infection. The COBAS TaqMan real-time HBV test, therefore, can detect serum HBV DNA in many patients with occult HBV infection. Indeed, in the present study, the HBV DNA level was 60, 126, and 98 copies/ml in the 3 patients in whom HBV DNA was detected.

In the present study that used a sensitive real-time PCR assay, the prevalence of occult HBV in serum was very low in HBsAg-negative patients in Japan, including those with and without HCV infection. This indicated a low association of circulating low-level HBV (one of the forms of occult HBV) with the development of HCC in patients with HBsAg-negative HCC. Thus, by evaluation with serum samples, we did not find evidence for an association between the circulating low-level HBV and the development of HCC in HBsAg-negative patients.

In a more recent report, the COBAS TaqMan HBV test has a high sensitivity for the detection of HBV DNA as well as high specificity [17]. However, further evaluation is needed to confirm the actual prevalence of circulating low-level HBV in HBsAg-negative patients with HCC using various detection methods for HBV DNA in serum. In addition, because 'occult HBV infection' contains the detection of HBV in liver tissue or HBV integration in HBsAg-negative patients, further investigations are needed to clarify the effect of entire 'occult HBV infection' as an etiological agent in hepatocarcinogenesis of HBsAg-negative patients, including the prevalence of the detection of HBV in liver tissue and the prevalence of integrated HBV using the various detection methods.

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Comparison of Hepatitis B Virus Subgenotypes in Patients With Acute and Chronic Hepatitis B and Absence of Lamivudine-Resistant Strains in Acute Hepatitis B in Japan

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Hepatitis B virus (HBV) has been classified into eight genotypes and can be further divided into several subgenotypes that have different geographic distributions. Because of increased human migration, the prevalence of rare subgenotypes is increasing in Japanese patients with acute hepatitis B. Lamivudine-resistant strains of HBV have begun to emerge in association with chronic hepatitis B. The aim of this study was to investigate the distribution of HBV subgenotypes and lamivudine-resistant strains in patients in Japan with acute hepatitis B. One hundred twenty-three patients with acute hepatitis B and 123 with chronic hepatitis B were studied. HBV subgenotypes and lamivudine-resistance mutations were determined by direct sequencing of the preS and polymerase region, respectively. HBV subgenotypes Aa (n = 3), Ae (n = 23), Ba (n = 7), Bj (n = 3), Cs (n = 7), Ce (n = 76), D (n = 2), and H (n = 2) were detected in patients with acute hepatitis. In patients with chronic hepatitis, HBV subgenotypes Ae (n = 4), Ba (n = 1), Bj (n = 18), and Ce (n = 100) were found. Non-common Japanese subgenotypes, that is, non-Bj and non-Ce, were detected more frequently in patients with acute hepatitis (35.8%) than in patients with chronic hepatitis (4.1%) (Odds ratio, 0.076; 95%CI, 0.029–0.200; $P < 0.0001$). Lamivudine-resistance mutations were detected in chronic hepatitis patients with breakthrough hepatitis but not in other patients. In conclusion, the prevalence of uncommon Japanese HBV subgenotypes is expected to increase, although lamivudine-resistant strains have not yet been found in patients with acute hepatitis B. *J. Med. Virol. 79:366–373, 2007.*

KEY WORDS: genotype; drug-resistance; phylogenetic analysis

INTRODUCTION

Approximately 350 million people worldwide are infected with hepatitis B virus (HBV) [Kao and Chen, 2002]. HBV infection causes chronic hepatitis and progresses to cirrhosis and hepatocellular carcinoma, which is the third most common cause of cancer-related death in Japan [Ganem and Prince, 2004; Kiyosawa et al., 2004]. Therefore, HBV infection is one of the most important global health problems, especially in endemic areas such as Asia. HBV has been classified into eight major genotypes on the basis of divergence of 8% in the full-length sequence [Okamoto et al., 1988; Norder et al., 2004]. Each genotype has a unique geographic distribution. Genotype A is found mainly in Europe, North America, and Africa. Genotypes B and C are predominant in East Asia. Genotype D is common in Mediterranean areas. However, several other groups recently reported that the occurrence of genotype A in

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cases of acute hepatitis B in Japan, where genotype A is rare, is increasing [Joh et al., 2003; Kobayashi et al., 2001; Yotsuyanagi et al., 2005; Takeda et al., 2006]. Therefore, the frequencies of other HBV strains that are rare in Japan may have increased among Japanese patients with chronic hepatitis B. HBV subgenotypes and their geographic distributions have also been reported [Norder et al., 2004]. HBV subgenotype Ba is found frequently in Asia, but subgenotype Bj has been found only in Japan [Sugauchi et al., 2003]. HBV subgenotype Ce is found in East Asia, including Japan and China, whereas subgenotype Cs occurs in Southeastern Asia [Huy et al., 2004, Norder et al., 2004; Chan et al., 2005; Tanaka et al., 2005]. Therefore, HBV subgenotypes can be used to study geographic distributions in greater detail than can simple genotypes. The first aim of the present study was to investigate HBV subgenotypes in patients in Japan with acute hepatitis B and to clarify the distribution of these subgenotypes.

Lamivudine has been used widely to treat chronic hepatitis B because it suppresses effectively viral replication, reduces disease activity, and improves liver histology. However, prolonged treatment with lamivudine has led to the emergence of drug-resistant strains. The underlying mutations occur in the HBV polymerase region. A lamivudine resistance mutation emerged in about 70% of patients who treated with lamivudine for 4 years [Hadziyannis et al., 2000; Liaw et al., 2000]. Lamivudine has been available since 2000, and lamivudine-resistant strains have been detected in patients in Japan with chronic hepatitis B. To date, acute hepatitis caused by a lamivudine-resistant strain has not been reported, and the clinical impact of lamivudine-resistant strains on acute hepatitis is not known. Thus, the second aim of the present study was to investigate the prevalence and clinical characteristics of lamivudine-resistant HBV strains in patients in Japan with acute hepatitis B.

MATERIALS AND METHODS

One hundred twenty-three Japanese patients with acute hepatitis B who were treated at Nagoya University Hospital or Ogaki Municipal Hospital were enrolled in this study. Patients were 88 men and 35 women, with a mean age of 38.6 ± 12.9 years (range, 16–75 years). The control group comprised 123 age- and sex-matched Japanese patients with chronic hepatitis B, including 10 patients with breakthrough hepatitis due to lamivudine-resistant strains. Acute hepatitis B was diagnosed as follows. Each patient had high titers of hepatitis B surface antigen (HBsAg) and IgM class antibody against HBV core antigen, elevated serum levels of alanine aminotransferase, and absence of antibodies against other causative viruses, such as hepatitis A virus, hepatitis C virus, Epstein-Barr virus, and cytomegalovirus. It was necessary to discriminate between development of chronic hepatitis after initial infection and acute onset of chronic infection. Thus, serum HBsAg levels noted in previous medical records pertaining to blood donation screening, labor and delivery screening, or employment health screening, for example, were obtained. Informed consent was obtained from all patients, and the study was carried out in accordance with the 1975 Helsinki Declaration.

Virologic Tests

HBsAg was measured with a commercially available kit (AxSYM HBsAg(V2); Abbott Japan, Tokyo, Japan). Antibody titers against hepatitis A virus and hepatitis C virus were measured with a commercial microparticle enzyme immunoassay (AxSYM HAVAB-M 2.0, AxSYM Anti-HCV; Abbott Japan).

HBV DNA was isolated from peripheral blood with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Nested polymerase chain reaction (PCR) analysis and direct sequencing of the preS, polymerase, and precore/core regions were performed as reported previously but with modifications [Allen et al., 1998; Lindh et al., 1999; Huy et al., 2004]. In brief, each 50 μl PCR reaction contained 100 nM each primer, 1 ng template DNA, 5 μl GeneAmp 10× PCR buffer, 2 μl dNTP, and 1.25 U AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primers for the preS region were sense (TCACCTATTCTTGGGAACAAGA) and antisense (GGCACTAGTAAACTGAGCCA); for the polymerase region were sense (CCTGCTGGTGGCTCCAGTTC) and antisense (GGTTGAGTCAGCAAACACACTTG); and for the precore–core region were sense (GTTGCATGGAGACCACCGTGAAC) and antisense (GTATGGTGAGGTGAACAATG). Amplification conditions consisted of 5 min at 94C followed by 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The second PCR was done in the same reaction buffer with the first-round PCR product as template and the following sets of primers: for the preS region, sense (TCACCTATTCTTGGGAACAAGA) and antisense (AGAAGATGAGGCATAGCAGC); for the polymerase region, sense (GGATGTGTCTGCGGCGTTT) and antisense (ACCCCATCTTTTTGTTTTGTTAGG); and for the precore–core region, sense (CTGACTACTAATTCCCTGGATGCTGGGTCT) and antisense (ATGTCGACAACCGACCTTGA). PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under Ultra Violet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). The neighbor-joining method [Saitou and Nei, 1987] was used for phylogenetic analysis of the preS region to classify HBV into subgenotypes. Bootstrap analysis (100 replicates) was performed. Sequences of the precore and core regions were used for discrimination between subgenotypes Ba and Bj, as reported previously [Sugauchi et al., 2003].

Statistical Analyses

Data are expressed as mean ± standard deviation (SD). Contingency table analysis with Fisher's exact probability test was used for comparisons between groups. $P < 0.05$ was considered statistically significant. The statistical software used was SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Distribution of HBV Subgenotypes

The results of phylogenetic analyses of HBV subgenotypes of the 123 patients with acute hepatitis B are shown in Figure 1. The following HBV subgenotypes were detected in patients with acute hepatitis B: Aa (n = 3), Ae (n = 23), Ba (n = 7), Bj (n = 3), Cs (n = 7), Ce (n = 76), D (n = 2), and H (n = 2). Subgenotype Aa was classified further as the Asia type in three patients. Two cases of subgenotype Ce were Okinawa variants. Results of phylogenetic analyses of HBV subgenotypes in the 123 patients with chronic hepatitis B are shown in Figure 2. In patients with chronic hepatitis, HBV subgenotypes Ae (n = 4), Ba (n = 1), Bj (n = 18), and Ce (n = 100) were found. One chronic hepatitis B patient infected with subgenotype Ae was a hemophiliac who received clotting factor concentrates from outside Japan. All cases of genotype B were confirmed by sequencing of the precore and core regions, and there were no differences between PreS and precore/core regions that allowed discrimination of Ba from Bj. Subgenotypes Bj and Ce, which were found frequently in chronic hepatitis B patients, were regarded as common Japanese subgenotypes, and subgenotypes Aa, Ae, Ba, Cs, D, and H, which were found rarely in chronic hepatitis B patients, were regarded as uncommon Japanese subgenotypes. These results are summarized in Table I. Uncommon Japanese subgenotypes were detected more frequently in patients with acute hepatitis B (35.8%) than in those with chronic hepatitis B (4.1%) (Odds ratio, 0.076; 95%CI, 0.029–0.200, $P < 0.0001$). Clinical characteristics of acute hepatitis B with uncommon Japanese subgenotypes and common Japanese subgenotypes are shown in Table II.

Distribution of Lamivudine-Resistant HBV Strains

Lamivudine resistance-associated mutations were detected within the HBV polymerase region (positions 464–562) by direct sequencing. Amino acid substitutions at position 552 (M to V or I) in the YMDD motif and at position 528 (L to M) were detected only in chronic hepatitis patients who suffered breakthrough hepatitis during lamivudine treatment, but not in other patients with acute or chronic hepatitis B.

DISCUSSION

Each HBV genotype has a unique geographic distribution; however, distributions of HBV genotypes are changing gradually due to the ease of international travel. In the United States, the prevalence of HBV precore and basal core promoter variants has increased due to the influx of immigrants from HBV-endemic countries [Chu et al., 2003]. Lamivudine has been used widely to treat chronic hepatitis B, and lamivudine-resistant strains have been reported [Hadziyannis et al., 2000; Liaw et al., 2000]. These changes in prevalence may be associated with differences in clinical course and responses to antiviral treatments. Therefore, a clear understanding of the prevalence of HBV genotypes and HBV strains is important for clinical management of HBV-related diseases. Therefore, the distribution of HBV subgenotypes and lamivudine-resistant HBV strains among patients in Japan with acute hepatitis B was investigated.

Subgenotypes Ce and Bj have accounted for the majority of HBV subgenotypes found in Japanese patients with chronic hepatitis B [Sugauchi et al., 2003; Huy et al., 2004; Norder et al., 2004; Chan et al., 2005; Tanaka et al., 2005; Takeda et al., 2006]. Genotype A has been reported in Japanese patients with chronic hepatitis B, but it is rare [Takahashi et al., 1998; Orito et al., 2001], and the origins of these strains have not been clear. In this study, HBV subgenotypes Ce and Bj together accounted for 118 of the 123 (95.9%) cases of chronic hepatitis B. Therefore, the original subgenotypes in Japan were Ce and Bj and regarded as common Japanese types, and the others identified in this study are uncommon Japanese types. Meanwhile, several recent studies [Joh et al., 2003; Kobayashi et al., 2001; Yotsuyanagi et al., 2005; Takeda et al., 2006] in Japan showed that the distributions of HBV genotypes differ between acute hepatitis B and chronic hepatitis B and that the prevalence of HBV genotype A is significantly higher among patients with acute hepatitis B than among those with chronic hepatitis B. The authors speculated that HBV genotype A, which was found in acute hepatitis B patients, originated outside Japan [Michitaka and Onji, 2004]. Genotypes B and C, which are predominant in patients with acute hepatitis B, also might be showed the same tendency as genotype A. Then genotypes B and C which origins are outside Japan would be increasing and be found in patients with acute hepatitis B. Thus, distribution of the subgenotypes for HBV genotypes B and C in patients with acute hepatitis B was examined to clarify the origin of the virus because HBV genotypes B and C include both common Japanese types (Bj, Ce) and uncommon Japanese types (Ba, Cs). Subgenotypes Ba and Cs were detected more frequently in acute hepatitis B patients (15.1%) than in chronic hepatitis B patients (0.8%) in this study (Odds ratio, 0.048; 95%CI, 0.006–0.371; $P < 0.0001$). Therefore, the trend noted for genotype A was also noted for genotypes B and C. These findings suggest that not only uncommon Japanese HBV genotype A but also uncommon Japanese HBV subgenotype Ba and Cs are becoming more common in Japanese patients with acute hepatitis B. Some attention should be paid to the existence of uncommon Japanese HBV subtypes among genotypes

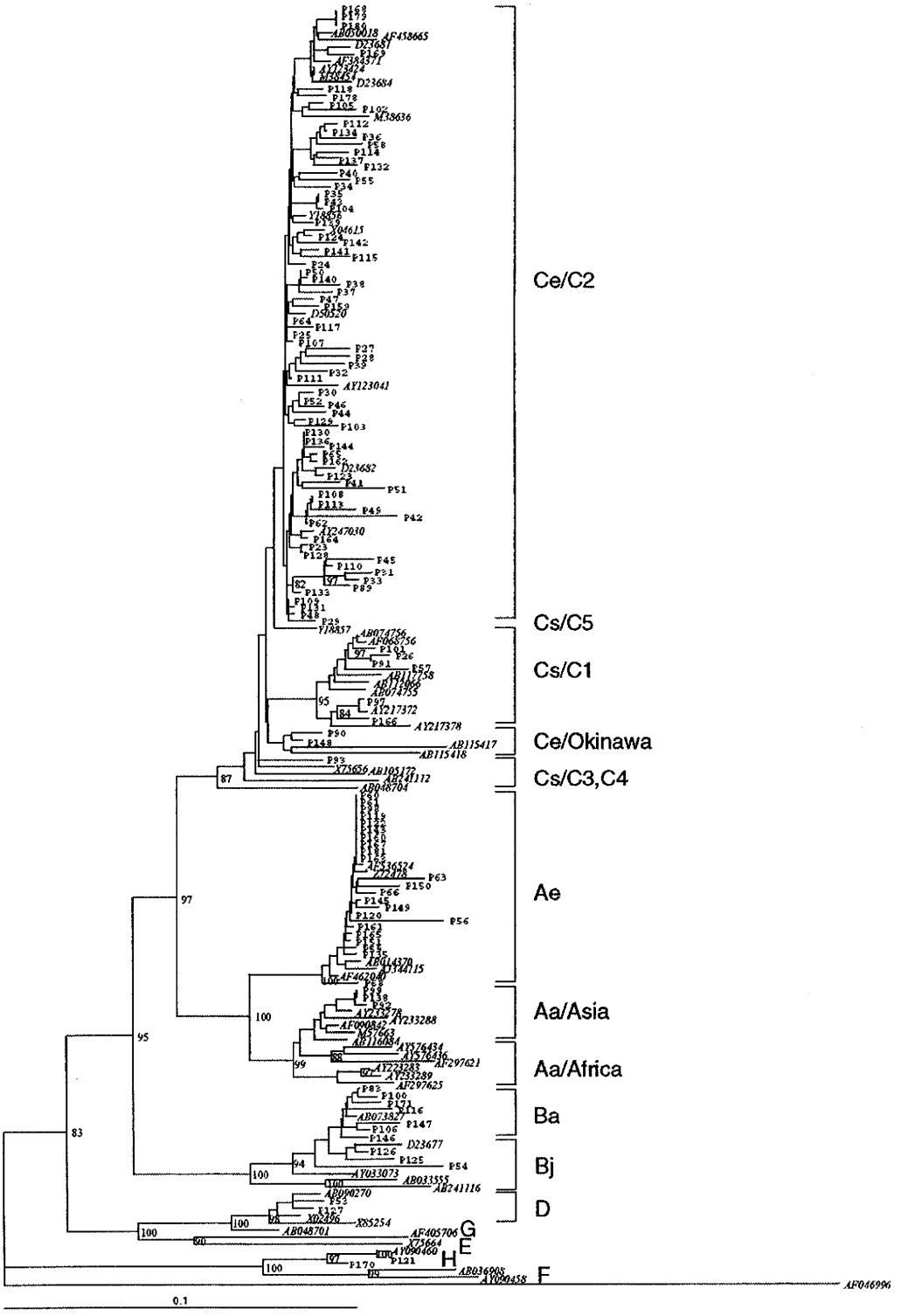

Fig. 1. Results of phylogenetic analysis of 123 sequences from the preS region of HBV of acute hepatitis patients and 59 reference strains from a database and shown by accession number. Phylogenetic analysis was performed by the neighbor-joining method with Woolly monkey HBV (AF046996) as out-group. Percentages of bootstrap values greater than 80% are shown on the nodes. The scale bar indicates genetic distance.

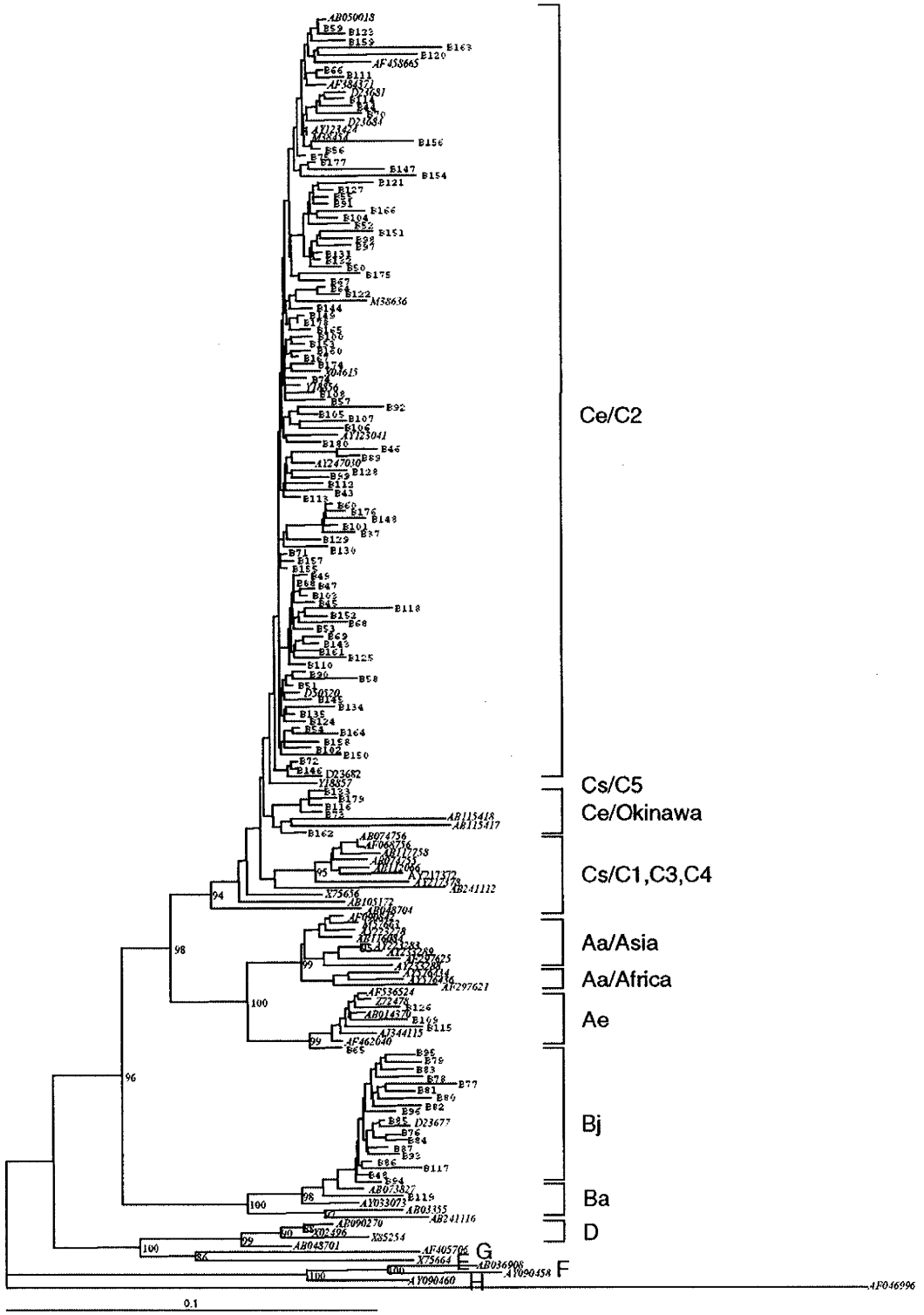

Fig. 2. Results of phylogenetic analysis of 123 sequences from the preS region of HBV of chronic hepatitis patients and 59 reference strains from a database and shown by accession number. Phylogenetic analysis was performed by the neighbor-joining method with Woolly monkey HBV (AF046996) as out-group. Percentages of bootstrap values greater than 80% are shown on the nodes. The scale bar indicates genetic distance.

TABLE I. Clinical Characteristics of 123 Acute Hepatitis B Patients and 123 Chronic Hepatitis B Patients

	Acute hepatitis (n = 123)	Chronic hepatitis (n = 123)	Odds ratio (95%CI)	*P*-value
Age	38.57+/−12.92	41.28+/−12.02		NS
Gender (male/female)	88/35	76/47	0.643 (0.377–1.098)	NS
HBV genotypes (Non-common Japanese types/ Common Japanese types)	44/79	5/118	0.076 (0.029–0.200)	0.0001

B and C, which are predominant types in Japan. A comparison between uncommon Japanese genotypes and common Japanese genotypes was undertaken. High-risk patients, such as those with multiple sexual partners and homosexuals, and patients who progressed to chronic hepatitis were found frequently to have uncommon Japanese genotypes. These results would be reflected with characteristics of genotype Ce which was majority in uncommon Japanese group and those of genotype Ae which was majority in uncommon Japanese group [Koibuchi et al., 2001; Takeda et al., 2006]. Virological and clinical differences between subgenotypes have been reported for genotypes B and C [Sugauchi et al., 2003; Chan et al., 2005]. The difference between HBV subgenotypes Ba and Bj was shown to be due to recombination with genotype C over the precore region and core gene [Sugauchi et al., 2003]. HBV subgenotype Ba leads more easily to development of hepatocellular carcinoma than does subgenotype Bj [Orito et al., 2005]. With respect to genotype C, C1858, which pairs with T1896 in the hairpin loop of the encapsidation sequence, was detected more frequently in subgenotype Cs (95%) than in subgenotype Ce (0%) [Chan et al., 2005]. The pairing of C1858 and T1896 is a stable structure and does not occur with A1896, which creates a TAG stop codon in the precore region. The presence of the A1896 mutation is associated with fulminant hepatitis [Omata et al., 1991]. These reported findings suggest clinical differences in acute hepatitis B between subgenotypes Cs and Ce. However, our sample size was too small to allow comparison of clinical differences in acute hepatitis B between HBV subgenotypes. Further studies are needed to clarify the influence of HBV subgenotypes on the clinical course of acute hepatitis B.

Lamivudine has been used widely to treat chronic hepatitis B, and lamivudine-resistant strains have been reported [Hadziyannis et al., 2000; Liaw et al., 2000]. As lamivudine use increases, the likelihood of lamivudine-resistant strains occurring in patients with acute hepatitis B increases. The prevalence of lamivudine-resistant strains in patients with acute hepatitis B and the clinical impact of lamivudine-resistant strains on acute hepatitis are unknown. Lamivudine has been used recently in patients with acute hepatitis to prevent the progression to fulminant hepatic failure or chronic hepatitis. Several studies have shown that lamivudine is safe and effective for treatment of acute hepatitis B [Kondili et al., 2004; Schmilovitz-Weiss et al., 2004]. Caution must be exercised in determining whether lamivudine should be used to treat acute hepatitis B because of the possibility of lamivudine-resistant strains. Multidrug-resistant strains of human immunodeficiency virus (HIV-1) are found in patients with primary HIV-1 infection, and this has become a serious problem [Brenner et al., 2004]. None of the patients with acute hepatitis B in the present study carried a lamivudine-resistant strain. The results of the present study indicate that lamivudine-resistant strains are not yet common among acute hepatitis B patients; therefore, lamivudine resistance is not a primary consideration in the treatment of acute hepatitis B at present. However, the possibility for acute hepatitis B caused by lamivudine-resistant strains exists. Moreover, as new antiviral drugs are developed, new drug-resistant strains will emerge. The prevalence of drug-resistant strains of HBV will increase in the future; therefore, surveillance to detect drug-resistant strains of HBV, including lamivudine-resistant strains, is important.

There are several reasons why lamivudine-resistant strains were not detected in acute hepatitis B patients in the present study. First, patients who received lamivudine also received education regarding prevention of

TABLE II. Clinical Features in Patients With Acute Hepatitis in Comparison With Common Japanese Types and Non-Common Japanese Types

	Non-common Japanese types (n = 44)	Common Japanese types (n = 79)	Odds ratio (95%CI)	*P*-value
Age	37.93+/−11.94	38.92+/−13.49		NS
Gender (male/female)	46/33	42/2	15.065 (3.404-66.667)	0.0001
Multiple sexual partners (Yes/No)	6/38	7/72	0.616 (0.193–1.962)	NS
Homosexual partners (Yes/No)	7/37	1/78	0.068 (0.008–0.571)	0.003
Progression to chronic hepatitis (Yes/No)	6/38	1/78	0.081 (0.009–0.699)	0.008
Progression to fulminant hepatitis (Yes/No)	2/42	7/72	2.042 (0.405–10.285)	NS

HBV infection so as to avoid new infection. Second, lamivudine-resistant strains did not have sufficient time to cause opportunity acute hepatitis. Chronic hepatitis B patients with breakthrough hepatitis caused by lamivudine-resistant strains were treated as soon as possible with interferon or adefovir dipivoxil [Perrillo et al., 2000, 2004; Suzuki et al., 2002] and, therefore, did not develop new infections. Third, lamivudine-resistant strains show weak infectivity and are not known to cause acute hepatitis. In vitro experiments showed that lamivudine-resistant strains had lower levels of viral replication than did wild-type strain [Melegari et al., 1998]. However, infectious experiments in an in vivo model are needed to prove this hypothesis [Wu et al., 2003]. New HBV subgenotypes and recombinants have been described recently, and therefore the prevalence of HBV strains causing acute hepatitis B will also change [Chen et al., 2004; Kurbanov et al., 2005; Wang et al., 2005]. The contributions of subgenotypes, recombinants, and lamivudine-resistant strains to the clinical features of acute hepatitis need to be clarified.

In conclusion, the prevalence of uncommon Japanese HBV subgenotypes appears to be increasing in Japan. Lamivudine-resistant mutations do not yet appear to be prevalent among patients in Japan with acute hepatitis B. Different HBV strains may have different clinical courses and responses to treatment; therefore, surveillance of HBV strains associated with acute hepatitis B will be useful for developing treatment protocols.

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Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection

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Table 1 Comparison of clinical factors between 642 anti-hepatitis C virus (HCV)-positive participants with and without clearance of serum HCV RNA

Clinical factors	Clearance of HCV RNA		p Value
	Yes	No	
Case numbers, n (%)	164 (25.5)	478 (74.5)	
Sex			0.449
Male, n (%)	56 (23.8)	179 (76.2)	
Female, n (%)	108 (26.5)	299 (73.5)	
Mean (SD) age (years)	56.4 (5.9)	56.4 (5.9)	0.987
Mean (SD) BMI (kg/m^2)	24.7 (3.3)	24.7 (3.8)	0.961
>27, n (%)	32 (22.5)	110 (77.5)	0.351
≤27, n (%)	132 (26.4)	368 (73.6)	
Mean (SD) ALT (IU/l)	27.8 (28.3)	64.2 (60.5)	<0.001
>34, n (%)	33 (9.7)	306 (90.3)	<0.001
≤34, n (%)	131 (43.2)	172 (56.8)	
HBsAg			<0.001
Positive, n (%)	34 (45.3)	41 (54.7)	
Negative, n (%)	130 (22.9)	437(77.1)	

ALT, alanine aminotransferease; BMI, body mass index; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

the cut-off value (= 1 SCO), in this study, the anti-HCV tests were also rechecked if the data were 1–2 SCO. For avoiding false-positive tests, anti-HCV was also rechecked for the participants with positive anti-HCV and negative HCV RNA. When comparing clinical factors between 642 anti-HCV-positive participants, with and without clearance of serum HCV RNA, we found that patients with positive HBsAg had a significantly higher proportion of HCV RNA clearance than those negative for HBsAg ($p<0.001$; table 1). In a stepwise logistic regression analysis, positive HBsAg was the only independent factor significantly associated with negative HCV RNA in anti-HCV participants (OR 0.348; 95% CI 0.211 to 0.574, $p<0.001$). HBV carriers were observed to have a significantly higher proportion of HCV RNA clearance than non-carriers among men and women (fig 1).

Viral interferences and reciprocal viral interactions have been observed between HBV and HCV dual infection.[7 8] Our previous study also showed that there might be a reciprocal viral interaction between HBV and HCV in patients with dual viral infection treated with interferon/ribavirin combination.[9] Reports from Egypt and Japan[2 3] were conducted in countries endemic for HCV where the prevalence of HBV carriers was <8% and they did not elucidate the influence the HBV infection. In our large-scale community-based study in an area endemic for HCV infection in a country hyperendemic for HBV, the important role of HBV carriers on HCV clearance was noteworthy and especially demonstrated. Besides, we did not find the effect of gender on the HCV clearance observed in previous studies.[2 3] It seems necessary to conduct prospective, longitudinal studies in clarifying roles of gender or concurrent HBV infection on the HCV clearance rate in patients infected with HCV.

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Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection

Alanine aminotransferase (ALT) activity is the most widely used laboratory parameter in the evaluation of necroinflammatory activity in liver disease.[1–3] However, it is incorrect to evaluate the arithmetic or the annual mean values. If the ALT level is high, the measurement interval decreases, whereas if the ALT level is low, the interval increases. As a result, the arithmetic mean value increases in patients with increased ALT levels. We performed a more accurate evaluation by using the integration value of ALT. The aim of this study was to determine the utility of the integration value of ALT in predicting hepatic carcinogenesis in patients with the hepatitis C virus (HCV) infection.

A total of 1704 consecutive patients with follow-up periods of ≥3 years, with no evidence of hepatocellular carcinoma (HCC) for ≥3 years before the observation period, and interferon treatment completed ≥3 year before the detection of HCC during the period from January 1995 to December 2002 were included. In all, 594 patients received interferon treatment and 1110 patients did not. All patients were followed up at Ogaki Municipal Hospital, Ogaki, Japan, at least every 6 months. During each follow-up examination, liver-function tests, including ALT, were measured. We calculated the area of a trapezoid with ALT value and the measurement interval, and added the values. We divided the integration value of ALT by the observation period to obtain the average integration value. Patients were classified into five groups according to the average integration value of ALT: group A, 0–20 IU/l (n = 217); group B, 21–40 IU/l (n = 614); group C, 41–60 IU/l (n = 446); group D, 61–80 IU/l (n = 240); and group E, ≥81 IU/l (n = 187). The median (range) follow-up period was 7.9(3.0–16.8) years. The total number of blood examinations was 90 211, and the median (range) number of blood examinations was 33 (6–222). Factors associated with the cumulative incidence of HCC were analysed using the Cox proportional hazard model with the forward selection method.

HCC occurred in 206 of 1704 (12.1%) patients during the follow-up period. According to univariate analysis, the following were significantly associated with the development of HCC:

- Age >65 years (relative risk (RR) 2.688 (95% CI 2.020 to 3.578); $p<0.001$);

Table 1 Factors associated with hepatocarcinogenesis (multivariate analysis)

	RR (95% CI)	p Value
Age (years)		
⩽65	1	<0.001
>65	1.964 (1.436 to 2.685)	
Sex		
F	1	0.001
M	1.675 (1.242 to 2.259)	
Average integration value of ALT (IU/l)		
0–20	1	<0.001
21–40	3.845 (1.117 to 13.298)	0.033
41–60	4.050 (1.206 to 13.597)	0.024
61–80	9.125 (2.789 to 29.857)	<0.001
⩾81	18.838 (5.735 to 61.881)	<0.001
Platelets (×104/mm^3)		
⩾12.0	1	<0.001
<12.0	3.277 (2.435 to 4.409)	
ALP (IU/l)		
⩽338	1	0.003
>338	1.590 (1.167 to 2.166)	
Cholinesterase (IU/l)		
⩾431	1	0.006
<431	7.856 (1.824 to 33.830)	
Albumin (g/dl)		
⩾3.5	1	<0.001
<3.5	2.901 (1.973 to 4.266)	
IFN treatment		
No treatment	1	0.015
Non-SVR	0.891 (0.429 to 1.851)	0.395
SVR	0.537 (0.349 to 0.827)	0.005

ALP, alkaline phosphatase; ALT, alanine aminotransferase; F, female; IFN, interferon; M, male; SVR, sustained virologicl response.

- Male sex (1.515 (1.138 to 2.018); p = 0.004);
- HCV genotype 2 (1.452 (1.003 to 2.102); p = 0.048);

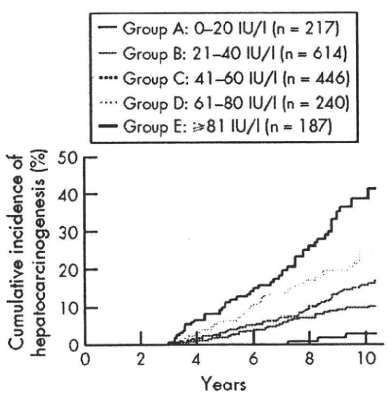

Figure 1 Incidence of hepatocarcinogenesis according to the average integration value of alanine aminotransferase (ALT). Patients were classified into five groups according to the average integration value of ALT: group A, 0–20 IU/l; group B, 21–40 IU/l; group C, 41–60 IU/l; group D, 61–80 IU/l and; group E, ⩾81 IU/l. With the exception of group B vs group C, group C vs group D and group D vs group E, the cumulative incidence of hepatic carcinogenesis differed significantly between the five groups (p = 0.0020 to <0.001).

- High average integration value of ALT (group B (4.977 (1.541 to 16.075); p<0.001); group C (8.598 (2.690 to 27.486); p<0.001); group D (14.989 (4.674 to 48.062); p<0.001); group E (25.358 (7.940 to 80.985); p<0.001), fig 1);
- High aspartate aminotransferase level >40 IU/ml (3.283 (2.272 to 4.745); p<0.001), low platelet count <12.0×10^4/mm^3 (5.214 (3.953 to 6.877); p<0.001);
- Low prothrombin time level ⩽70% (2.575 (1.760 to 3.768); p<0.001);
- High γ glutamyl transpeptidase level >56 IU/ml (2.615 (1.990 to 3.438); p<0.001);
- High total bilirubin level >1.2 mg/dl (1.990 (1.279 to 3.098); p = 0.002);
- High alkaline phosphatase level >338 IU/ml (3.126 (4.604 to 74.783); p<0.001);
- Low cholinesterase level <431 IU/ml (18.555 (4.604 to 79.783); p<0.001);
- High total protein level ⩾6.5 g/dl (1.775 (1.065 to 2.958); p<0.001);
- Low albumin level <3.5 g/dl (4.881 (3.341 to 6.945); p<0.001);
- Low total cholesterol level <130 mg/dl (11.925 (1.671 to 85.085); p<0.001);
- Type of response to IFN treatment (sustained virologic response, 0.142 (0.074 to 0.273) p<0.001; non-sustained virologic response, 0.403 (0.260 to 0.601); p<0.001).

According to multivariate analysis, increasing age, male sex, low platelet count, high average integration value of ALT, low cholinesterase level, low albumin and type of response to interferon treatment were significantly associated with the incidence of HCC as shown in table 1.

We showed that increased liver inflammation, as assessed by increased ALT level, is associated with increased risk for development of HCC in patients with HCV infection.[4–6] The average integration value of ALT, even within the current normal range, was strongly associated with the cumulative incidence of hepatocarcinogenesis. Inhibition of ALT to a value as low as possible is necessary for the prevention of hepatic carcinogenesis.

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BOOK REVIEWS

Upper Gastrointestinal Surgery

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In the rapidly developing field of upper gastrointestinal surgery, there is often a need for access to short synopses of areas of development without the need to resort on every occasion to a literature search or standard text. This concise book brings together a selection of internationally acknowledged experts to provide such a text. The areas covered provide a rapid review of topics which would be of particular interest to specialist

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HEPATOLOGY

Efficacy of ribavirin plus interferon-α in patients aged ≥60 years with chronic hepatitis C

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age group, hepatitis C virus, interferon, ribavirin, sustained virologic response.

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Abstract

Background: In Japan, patients with hepatitis C virus (HCV)-associated liver disease are getting older, and thus the number of deaths due to such disease is increasing. The efficacy of combination therapy with ribavirin and interferon for chronic HCV infection in elderly patients has not been fully clarified. The aim of the present study was to evaluate the efficacy and tolerability of combination therapy in such patients.

Methods: Two hundred and twenty consecutive patients with chronic hepatitis C were treated with combination therapy. These patients were divided into two groups according to age: patients ≥ 60 years ($n = 66$) and patients < 60 years ($n = 154$). Clinical characteristics, the sustained virologic response (SVR) rate obtained by intention-to-treat analysis, and the rate of reduction or discontinuation of ribavirin were compared between the two groups.

Results: The ribavirin discontinuation rate was significantly higher in the patients aged ≥60 years than in the patients aged <60 years. However, the SVR rates did not differ significantly between patients aged ≥60 years and those aged <60 years (31.8% *vs* 38.3% by intention-to-treat analysis). According to multivariate analysis, genotype and HCV viral load were significantly associated with SVR while patient age did not affect SVR.

Conclusions: Treatment of chronic hepatitis C with combination therapy was comparably effective between patients aged ≥60 years and those aged <60 years, although the ribavirin discontinuation rate was higher among the older patients than the younger patients.

Introduction

Hepatitis C virus (HCV) infection is a widespread viral infection that often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The need for treatment of chronic HCV infection in the elderly is increasing in Japan and is expected to increase in the USA and other Western countries.[1]

Sustained virologic responders who are negative for serum HCV-RNA 6 months after treatment with interferon (IFN) are reported to be likely to remain in virologic and biochemical remission with histologic improvement.[2,3] Moreover, IFN therapy reduces the risk of hepatocellular carcinoma among virologic or biochemical responders.[4–6] Ribavirin is now generally used in combination with IFN for the treatment of chronic hepatitis C, and this therapy has been reported to be more effective than IFN monotherapy, with a higher rate of HCV eradication.[7–10]

Efficacy of IFN monotherapy in elderly patients with chronic hepatitis C has been reported,[11,12] but efficacy of combination ribavirin and IFN therapy in elderly patients has not been established. We retrospectively evaluated the efficacy and tolerability of ribavirin plus interferon in patients aged ≥60 years with chronic hepatitis C.

Methods

Patients

Two hundred and twenty consecutive patients with chronic hepatitis C with a high viral load (we defined high viral load as serum HCV-RNA level >100 KIU) were treated with IFN and ribavirin in combination between January 2002 and April 2003 at 14 institutions: Nagoya University Hospital and affiliated hospitals. One hundred and twenty-two of 220 patients were naïve patients. All met the following inclusion criteria: <75 years old; positivity for anti-HCV antibody; and serum HCV-RNA level > 100 KIU/mL on

Table 1 Patients treated by combination therapy

	Total patients (n = 220)	Age <60 years (n = 154)	Age ≥60 years (n = 66)	P
Sex ratio (male/female)	147/73	109/45	38/28	0.0567
Baseline serum ALT (IU/L)	94.0 ± 68.6	92.4 ± 71.4	97.6 ± 62.0	0.6081
Hemoglobin (g/dL)	14.3 ± 1.3	14.5 ± 1.3	13.9 ± 1.4	0.0056
Creatinine clearance (mL/min)	101.6 ± 24.5	106.5 ± 24.6	85.3 ± 15.2	<0.0001
Genotype (1/2/other)	169/50/1	115/38/1	54/12/0	0.4510
HCV-RNA (KIU/mL)	648.7 ± 339.4	638.8 ± 342.3	671.9 ± 333.9	0.5090
Activity (A0/A1/A2/A3)	6/77/63/18	5/55/44/14	1/22/19/4	0.8405
Fibrosis (F0/F1/F2/F3/F4)	8/74/45/26/10	6/54/34/17/7	2/20/11/9/3	0.9199

ALT, alanine aminotransferase; HCV-RNA, hepatitis C virus RNA.

quantitative polymerase chain reaction (PCR) assay (Amplicor Monitor Assay; Roche Molecular Systems, Pleasanton, CA, USA) within 12 weeks preceding the therapeutic period. Exclusion criteria included pretreatment hemoglobin level < 10 g/dL, positivity for serum hepatitis B surface antigen, drug addiction, alcohol abuse, autoimmune hepatitis, primary biliary cirrhosis, coexisting serious psychiatric or medical illness, and pregnancy. To exclude any patient bias, only complete cohorts from each hospital were enrolled. HCV genotypes were determined by PCR with genotype-specific primers.[13,14]

All patients were treated with 6–10 MU IFN-α-2b (Intron A, Schering Plough, Osaka, Japan) daily for 2 weeks, followed by the same dose of IFN three times a week for 22–46 weeks. We conducted 24 weeks of treatment at first. In the last 44 patients treatment duration was elongated to 48 weeks because this produced higher efficacy than 24 weeks of treatment. Oral ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ, USA) was administered for 24 weeks at 600 mg/day for patients who weighed ≤60 kg and at 800 mg/day for those who weighed >60 kg during the treatment period. The dose of ribavirin was reduced by 200 mg/day when the patient's hemoglobin concentration fell below 10 g/dL because of hemolytic anemia induced by the drug. Ribavirin was discontinued when IFN therapy was discontinued. In Japan, combination with interferon and ribavirin therapy was approved for medical insurance coverage in 2001 with a limit in ribavirin administration of up to 24 weeks. Combination therapy with peg-interferon and ribavirin was not approved for medical insurance coverage in Japan until November 2004.

Liver histology

Pretreatment liver biopsy specimens were classified in terms of fibrosis on a scale of F0–F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis) and in terms of necroinflammatory activity on a scale of A0–A3 (A0, no histologic activity; A1, mild activity; A2, moderate activity; A3, severe activity).[15]

Assessment of efficacy

Virologic response was assessed by qualitative HCV-RNA assay with a lower sensitivity limit of 100 copies/mL (Amplicor HCV version 2.0; Roche Molecular Systems). According to the qualitative HCV-RNA results, responses were defined as follows: sustained virologic response (SVR), no HCV-RNA detected at the end of the 24-week follow-up period after completion of treatment; relapse, no HCV-RNA at end of treatment and reappearance of serum HCV-RNA during the 24 week follow-up period; or non-response (NR), persistent positive serum HCV-RNA throughout treatment.

Comparison of characteristics and efficacy of treatment according to age

Patients were divided by age into two groups: those aged ≥60 years (n = 66) and those aged <60 years (n = 154). Sex ratio, baseline serum alanine aminotransferase level, pretreatment hemoglobin level, creatinine clearance, HCV genotype and viral load, histologic activity and fibrosis were compared between the two groups (Table 1). End-of-treatment virologic response (ETR) rate and SVR rate obtained by intention-to-treat analysis and per-protocol analysis, and the rate of reduction or discontinuation of ribavirin were compared between the two groups (Table 2).

Comparison of treatment efficacy between combination therapy and monotherapy in older patients

We examined efficacy of combination therapy in comparison to that of monotherapy in patients aged ≥60 years. For this purpose, we included as historical controls 257 patients with chronic hepatitis C with a high viral load treated with IFN-α alone. These were 168 men and 89 women aged 18–69 years (mean ± SD, 50.1 ± 9.9 years) treated at Nagoya University Hospital or Ogaki Municipal Hospital from 1989 to 2001. Forty-seven patients out of 257 were >60 years. All patients were treated with 6–10 MU IFN-α daily for 2 weeks, followed by the same dose of IFN-α three times a week for 22–46 weeks.

The study protocol was approved by the ethics committee of each hospital, and written informed consent was obtained from each patient before therapy.

Statistical analysis

Values are expressed as mean ± SD. Between-group differences in mean quantitative values were analyzed using Student's t-test, and differences in non-parametric data were analyzed by Mann–Whitney U-test. Differences in proportions were tested using χ^2 test. Multiple logistic regression analysis was used to identify factors related to SVR. All statistical analyses were performed

Table 2 Efficacy of combination therapy

	Total patients ($n = 220$) % (n)	Age <60 years ($n = 154$) % (n)	Age ≥60 years ($n = 66$) % (n)	P
SVR rate (intention-to-treat)	36.4 (80/220)	38.3 (59/154)	31.8 (21/66)	0.3589
SVR rate (per-protocol)	43.7 (80/183)	45.0 (59/131)	40.4 (21/52)	0.5671
ETR rate (intention-to-treat)	71.8 (158/220)	71.4 (110/154)	72.7 (48/66)	0.8444
ETR rate (per-protocol)	81.4 (149/183)	79.4 (104/131)	86.5 (45/52)	0.2621
SVR/relapse/NR/discontinuation	80/69/34/37	59/45/27/23	21/24/7/14	0.2834
Ribavirin discontinuation rate	24.5 (54/220)	20.8 (32/154)	33.3 (22/66)	0.0474
Ribavirin dose reduction rate	33.6 (74/220)	29.9 (46/154)	42.4 (28/66)	0.0709
IFN discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540
IFN dose reduction rate	15.9 (35/220)	15.6 (24/154)	16.7 (11/66)	0.8406
Combination therapy discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540

ETR, end of treatment virologic response; IFN, interferon; NR, non-response; SVR, sustained virologic response.

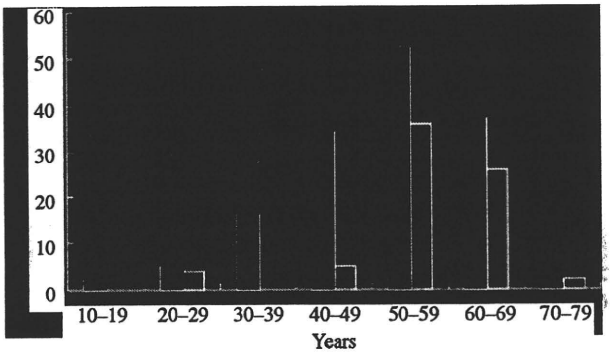

Figure 1 Patient age distribution by decade. (■) Male; (□) female.

using SAS software (SAS Institute, Cary, NC, USA). All P were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Patients were 147 men and 73 women aged 17–71 years (mean ± SD, 53.0 ± 11.1 years). The age distribution of patients treated with combination therapy is shown in Fig. 1. Patients ≥ 60 years comprised 30.0% of the patient population (66/220). The majority of female patients were over age 50 years (87.7%, 64/73). Clinical characteristics of the two study groups are shown in Table 1. The hemoglobin level was significantly lower in patients aged ≥60 years than in patients aged <60 years ($P = 0.0056$). Creatinine clearance in patients aged ≥60 years was worse than that in patients aged <60 years ($P < 0.0001$).

Response to therapy

The ribavirin discontinuation rate was significantly higher in patients aged ≥60 years than in patients aged <60 years ($P = 0.0474$). The dose ribavirin reduction was higher in the patients aged ≥60 years, but the difference did not reach statistical significance (42.4% *vs* 29.9%; $P = 0.0709$). However, the IFN discontinuation and dose reduction rate did not differ significantly between the two groups. The treatment discontinuation rate did not differ significantly between the two groups. As a result, the SVR rate by both intention-to-treat analysis and per-protocol analysis did not differ significantly between the two groups. And ETR rate by both intention-to-treat analysis and per-protocol analysis also did not differ significantly between the two groups (Table 2).

Histologic factor associated with SVR were determined by univariate analysis. The SVR rate of the F0–1 patients was not different from that of the F2–4 patients (49.3% *vs* 47.7%, $P = 0.8490$ by per-protocol analysis; 43.9% *vs* 38.3%, $P = 0.4651$ by intention-to-treat analysis). Factors associated with SVR in combination therapy were determined by multivariate analysis (Table 3). Genotype ($P < 0.0001$, odds ratio 0.074, 95% confidence interval [CI]: 0.030–0.182), and viral load ($P = 0.0002$, odds ratio 1.002, 95%CI: 1.001–1.004]) were significantly associated with SVR, but age was not significantly associated with SVR.

Clinical characteristics of the 66 patients aged ≥60 years who underwent combination therapy and 47 historical control patients aged ≥60 years who underwent monotherapy are shown in Table 4. The SVR rate with combination therapy was significantly higher than that with monotherapy (31.8%, 21/66 *vs* 10.6%, 5/47, $P = 0.0084$ by intention-to-treat analysis; 40.4%, 21/52 *vs* 10.6%, 5/47, $P = 0.0008$ by per-protocol analysis). Treatment discontinuation rate of combination therapy tends to be higher than that of monotherapy, but there was no significant difference between the two groups. This is because the number of patients undergoing monotherapy was small.

Virologic response to combination therapy and to IFN monotherapy in patients with HCV genotype 1 and a high viral load are shown by age group in Fig. 2.

With monotherapy, the SVR rate decreased with age, but with combination therapy, the SVR rates of patients in their 40s, 50s, and 60s and higher were similar. In patients ≥60 years with genotype 1 and a high viral load, the SVR rate with combination therapy was significantly higher than that with monotherapy (27.5% *vs* 6.7%, $P = 0.0322$ by per-protocol analysis).

Virologic responses to combination therapy and to IFN monotherapy in patients with HCV genotype 2 and a high viral load are shown by age group in Fig. 3.