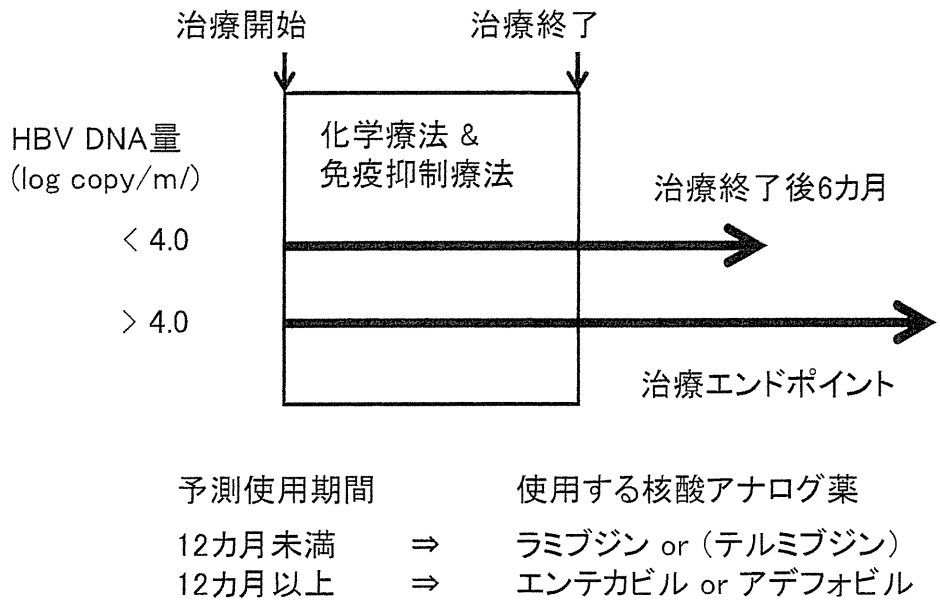

Figure 2. 非活動性キャリアにおけるB型肝炎再活性化の対策（文献18から改変）.

いた免疫力が回復し強いウイルス排除反応，すなわち肝炎の再活性化がおこる．再活性化したB型肝炎は重症化・劇症化し致死的になることもまれではない．このため，キャリアで化学療法薬や免疫抑制薬を使用する場合は再活性化に対する注意が必要であり，最近では核酸アナログ薬による予防が積極的に行われるようになっている．

B型肝炎再活性化の頻度はHBVの感染状態と免疫抑制の程度により異なる．HBVキャリアでは，免疫抑制の程度が比較的弱くても20～50%に再活性化がおこり，造血幹細胞移植や肝移植では50%を超える再活性化がおこりさらに危険性が高い．悪性腫瘍の化学療法にともなう再活性化の報告には悪性リンパ腫，乳癌，多発性骨髄腫，肺癌，膵癌などの報告があるが，報告が多いのは悪性リンパ腫と乳癌である．膠原病などの免疫抑制療法にともなう再活性化ではステロイドによるものが多い，最近はTNFα阻害薬などの分子標的薬による再活性化の報告が増加している[17]．

IV 非活動性キャリアにおける再活性化対策

アメリカ肝臓学会が2007年に発表したB型慢性肝炎のガイドラインの中に，化学療法・免疫抑制療法にともなうB型肝炎再活性化対策がある(Figure 2)[18]．対策としては核酸アナログ薬の予防投与が基本であり，化学療法・免疫抑制療法開始前より投与を開始する．核酸アナログ薬は化学療法・免疫抑制療法中は継続して投与する必要があり，治療が終了してもすぐには中止しない．中止時期は治療開始前のウイルス量により異なっており，血中HBV DNA量が4.0log copy/m*l*以下であれば化学療法・免疫抑制療法終了後6カ月で核酸アナログ薬を中止する．一方，ウイルス量が4.0log copy/m*l*以上であれば通常の慢性B型肝炎の治療に準じて治療を継続する必要がある．使用薬物は，予防投与期間が12カ月以内と予測される場合はラミブジンまたはテルビブジン（日本では未承認）が，またこの期間が12カ月以上と予測される場合は耐性株が出現しにくいエンテカビルまたはアデフォビルが推奨されている．日本でのB型慢性肝炎治療のガイドラインではエンテカビルが第一選択であり，再活性化の予防についてもエンテカビルが選択される傾向にある．インターフェロンは骨髄抑制の副作用があるため使用しない．

Hsuら[19]は非ホジキンリンパ腫の患者を対象として，ラミブジンを予防的に使用した場合と肝炎発症後の治療に使用した場合を前向きに比較検討した．この結果，ラミブジンの予防投与はB型

肝炎の再活性化の頻度や重症度を有意に低下させることを報告した．Saab ら[20]は Decision Analysis Model を用いて，HBV キャリアの悪性リンパ腫の患者に対してラミブジンを予防的に内服させることは費用対効果が高いことを報告した．さらに，ラミブジン投与群では原疾患による死亡数も減少することが予測されている．これは，ラミブジン投与によりB型肝炎の再活性化がおこらなければ，抗腫瘍薬などの休薬による原疾患加療の中断がないためと考えられている．このように，HBV キャリアにおける再活性化対策として核酸アナログ薬の予防投与が有用であり，これらの成績を基に対策が立てられた．予防投与の期間は，化学療法終了後2カ月までででは不十分であり，基本的に6カ月までの投与が推奨されている．

V　De novo B 型肝炎

HBs 抗原陰性で HBc 抗体 and/or　HBs 抗体陽性者，すなわち HBV の既往感染者は本邦の人口の約 1/5 を占める．以前は，既往感染状態になると HBV は宿主から完全に排除されていると考えられていた．しかし，その後の検討で，HBV 遺伝子が cccDNA の形で核内に残存しごく低レベルで複製していることが明らかになった．HBV に感染すると，非特異的な自然免疫に引き続きウイルス特異的な免疫反応がおこる．この時，細胞障害性 T 細胞（CTL）による細胞性免疫がウイルス排除に重要な役割を果たしている[21]．この細胞性免疫反応は急性肝炎治癒後，年単位で持続する．これは，肝細胞内で感染性のあるウイルスの産生が持続していることを示すが，CTL の働きにより血中へのウイルスの放出は極めて少量に抑えられている．これが HBs 抗原陰性で既往感染とされる場合の感染状態である．

宿主の免疫能が低下し，特異的 CTL がウイルスの増殖をコントロールできない状態になると HBV の増殖が盛んになりB型肝炎が再燃する．この状態が de novo B 型肝炎である．化学療法後の de　novo　B 型肝炎の報告は 1975 年の Wands ら[22]によるものが最初と思われる．しかし，その当時は HBV の潜伏感染に関する知識に乏しく，その臨床的意義は不明であった．1991 年，Lok ら[23]は化学療法を受けた悪性リンパ腫患者についてB型肝炎の再活性化を前向きに調査した．この結果，HBV キャリアでの再活性化は高頻度であったが，de novo B 型肝炎の発症は 2% 程度とまれであることを報告した．これに対し，血液疾患にともない同種骨髄移植を施行した症例では de　novo　B 型肝炎の発症は 14～50% と比較的高率であることが報告されている．この両者の頻度の差は，おそらく免疫抑制の程度が骨髄移植では通常の化学療法よりも強いためと考えられる．

1998 年，Uemoto ら[24]は生体肝移植症例における de novo B 型肝炎を検討し，ドナーが HBV の既往感染の場合，移植後 94%（15/16）のレシピエントに de novo B 型肝炎を発症したことを報告した．さらに，この報告ではドナー肝の HBV とレシピエントから分離された HBV の塩基配列が一致することを確認し，レシピエントの HBV がドナー由来であることを証明した．その後，肝移植後の de　novo　B 型肝炎に関する報告は多く[25)～33)]，その特徴をまとめると以下のようになる．発症時期は移植後しばらくして経過してからであり，中央値は 12 カ月と長い．ドナーが既往感染者の場合の感染率は高く，33～96% と報告されている．肝炎を発症すると HBV は高率に持続感染化する．肝炎の重症度は症例により大きく異なり予測は困難である．レシピエントが HBs 抗体陽性でも感染は成立する．しかし，HBIG（hepatitis B immunoglobulin）または HB ワクチンで HBs 抗体価を保つことにより予防可能である．

近年，de novo B 型肝炎が肝臓の専門家だけでなく血液の専門家の間でも注目を浴びるようになった．その理由は，悪性リンパ腫の治療に抗 CD20 抗体であるリツキシマブが使用されるようになったためである．リツキシマブの使用で悪性リンパ腫の治療成績は大きく改善したがB型肝炎再活性化の頻度も高くなった．リツキシマブの使用によって de novo B 型肝炎を発症した症例が 2001 年に Dervite ら[34]により初めて報告された．すなわち，HBs 抗原陰性，HBs 抗体陽性の患者がリツキシマブを含む化学療法施行後に HBs 抗原と HBV　DNA が陽性となり劇症肝不全をおこ

して死亡したという症例である．その後もリツキシマブ使用と関連したde novo B型肝炎例が報告され注目を集めた．

香港のHuiら[35]は，悪性リンパ腫の治療を受けた244名を対象に前向きコホートでde novo B型肝炎，劇症肝不全の発症率およびそれらの危険因子を検討した．彼らの報告では244例中8例（3.3%）でde novo B型肝炎を発症しており，その内3例で劇症肝不全を発症し1例が死亡した．De novo B型肝炎発症の危険因子はリツキシマブとステロイドの併用であった．さらに，de novo B型肝炎の発症は劇症肝不全を引きおこす唯一の危険因子であった．香港のYeoら[36]も104例の悪性リンパ腫症例を対象にde novo B型肝炎の発症を検討した．HBs抗原陰性でHBc抗体陽性の既往感染者が104例中46例あり，この46例中R-CHOP療法を受けた21例では4例（24%）でde novo B型肝炎を発症したのに対し，CHOP療法を受けた25例ではde novo B型肝炎の発症はなかった（P＝0.015）．これらの成績から，リツキシマブの使用が有意にde novo B型肝炎の発症に関連していることは明らかである．

近年，白血病などの治療に骨髄幹細胞移植が広く用いられるようになり，これにともなうde novo B型肝炎の報告も増えている[37)~45)]．骨髄幹細胞移植での特徴は，悪性リンパ腫のR-CHOP療法と比較するとde novo B型肝炎の発症時期が遅い症例が多いことである．すなわち，R-CHOP療法後は中央値で3カ月（範囲0～9カ月）であるのに対し，骨髄幹細胞移植後は中央値で16カ月（範囲3～25カ月）と長い傾向にある．これは，骨髄幹細胞移植では長期に免疫抑制薬を使用することが主な要因と考えられる．

インフリキシマブやエタネルセプトなどのTNFα阻害薬は，近年，関節リウマチやクローン病の治療に広く用いられるようになった．TNFα阻害薬もHBVの増殖を活性化することが知られており，非活動性キャリアからの再活性化に加えde novo B型肝炎の報告もみられる[46)~51)]．特徴としては，病歴が長く，本薬に加えグルココルチコイドなどの再活性化を促進する薬物も併用されていることが多い．短期での発症は少ないことが予測され[52]，長期に使用する場合は注意が必要である．

VI 本法でのde novo B型肝炎の実態調査

本邦ではde novo B型肝炎のまとまった疫学的なデータがなかったため，平成17年度の厚生労働省研究班（熊田博光班長）で全国調査を行った[53]．この調査では，5年間に新たにHBs抗原陽性になった患者として，B型急性肝炎1184例とde novo B型肝炎55例（4.4%）が登録された．劇症化率はde novo肝炎で27%，急性肝炎で7%であり，de novo肝炎で有意に高い傾向がみられた．また，劇症化例の死亡率はde novo肝炎で100%，急性肝炎で44%であり，これもde novo肝炎で有意に高い傾向がみられた．すなわち，これらの成績は，一旦de novo B型肝炎を発症して劇症化すると救命は非常に困難であることを示す．さらに，肝炎発症後に核酸アナログ薬を投与しても劇症化が回避できないことがわかり，早期の核酸アナログ薬投与の重要性が示された．

De novo B型肝炎が重症化しやすい要因はいくつか考えられる．第一は，通常のB型急性肝炎に比較して年齢が高いことである（中央値：63歳vs. 33歳，P＜0.001）．約30歳の差があり，明らかにde novo肝炎患者で不利である．第二は基礎疾患の有無であり，de novo肝炎患者の多くは背景に重篤な疾患を持ち，肝炎発症前から全身状態が不良である．第三は，すでに数々の治療が行われており，薬物性肝障害などの他の肝障害をおこす要因があることから，肝炎を発症してもde novo B型肝炎と認識されずに対応が遅れることが多いことが挙げられる．

VII De novo B型肝炎の対策

Table 3に，現在実施または検討されているde novo B型肝炎対策を整理した．HBIGやHBワクチンによるHBs抗体価の維持は移植後のde novo B型肝炎の予防に用いられている．具体的には肝移植時からHBIGを投与しHBs抗体価を維持する方法である[24]．通常，その後の経過でHBワクチンを併用しHBs抗体価を維持する．信州大学での検討では，90%以上の症例で最終的に

Table 3. B型肝炎の再活性化対策

1. HBIGやHBワクチンによるHBs抗体価の維持.
2. 核酸アナログ薬の予防投与.
3. 血中HBV DNAの定期検査による早期発見.

HBワクチンのみでHBs抗体価の維持が可能になっている．最初に核酸アナログ薬の予防投与を行い，その後HBワクチンでHBs抗体価を維持する施設もある．

核酸アナログ薬の予防投与はde novo B型肝炎の予防に有効であると考えられている．また，同薬は副作用が少なく経口薬であることから使いやすい薬物である．しかし，薬価が高くde novo B型肝炎の予防に保険適応がないことが最大の問題点である．今後，核酸アナログ薬の予防投与を一般化するためには解決すべき課題は多い．その中で，de novo B型肝炎を発症しやすい条件をさらに詳細に解明し，費用対効果を向上させることが特に重要と考えられる．

De novo B型肝炎対策として定期的に血中のHBV DNAを測定し，HBVの再増殖がみられた時点で核酸アナログ薬を投与する方法が提案されている．この方法の基になった研究がHuiら[35]の報告である．彼らはde novo肝炎患者を詳細に検討した結果，再活性化する症例ではまずHBV DNAが血中で陽性となり，それから中央値で10週後にHBs抗原が陽転化し，さらに中央値で18週後にALT値が上昇して肝炎が再燃することを明らかにした．このように，血中HBV DNAが陽性になってから肝炎を発症するまでの期間は十分長いので，血中HBV DNA量を定期的に検査し，前値の100倍に増加した時点で核酸アナログ薬を投与する予防策を提案をした．これに対し，Liuら[54]は核酸アナログを予防的に内服させた方が費用の面で効果的ではないかと反論している．

日本でのde novo B型肝炎に対する対策は厚生労働省の班会議を中心に進められている．鹿児島大学の坪内教授を中心として作られた「免疫抑制・科学療法により発症するB型肝炎対策ガイドライン」は日本で最初の指針である[55]．このガイドラインの要点は血中HBV DNAを免疫抑制・化学療法終了後少なくとも12カ月間，毎月測定し，HBVの再増殖がみられた時点で核酸アナログ薬を投与する方法である．また，この対策を前向きコホートで検証する研究が名古屋市大の楠本先生を中心としたグループなどで行われており，血中HBV DNAをモニターする方法の有効性が確認されつつある．

おわりに

以上，B型肝炎の再活性化について述べた．医療の進歩にともない，今後も新しい免疫抑制薬や化学療法薬が開発され，さらにこれらの薬剤を使用する機会も増えると予測される．B型肝炎再活性化に対する対策はその検討が開始されたばかりで，現状では十分とはいえない．この肝炎をなくすためには，今後さらなる基礎および臨床研究が必要である．

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CORRESPONDENCE

Pretreatment Prediction of Virological Response to Peginterferon Plus Ribavirin Therapy in Patients with Chronic Hepatitis C, Using Viral and Host Factors: Some Concerns

To the Editor:

We read with great interest the article published in a recent issue of HEPATOLOGY.[1] Shirakawa et al. enrolled 120 patients with chronic hepatitis C (CHC) infected with genotype 1 hepatitis C virus (HCV-1) and high baseline viral loads (HVL), defined by HCV RNA levels $\geq 10^5$ international units (IU)/mL measured by quantitative Cobas Amplicor assays (Roche Diagnostics Co. Ltd, Tokyo, Japan), who underwent combination therapy scheduled for 48 (85%) or 72 (15%) weeks. The authors concluded that the sequence of interferon sensitivity determining region of the HCV, the T-helper type 1 and type 2 (Th1/Th2) ratio, body weight, and neutrophil count can be useful for accurately predicting actual sustained virologic response (SVR) rate before combination therapy.

The definition of HVL ($\geq 10^5$ IU/mL) by the authors is not in accordance with the Asian Pacific Association for the Study of the Liver consensus statements ($\geq 4 \times 10^5$ IU/mL)[2] or other studies ($\geq 8 \times 10^5$ IU/mL).[3,4] Whether this definition influences the results deserves further study. The rates of rapid virologic response (RVR, defined as HCV RNA negative at week 4) and SVR were 45% and 21.7%, respectively, in their study. We examined the 200 Taiwanese patients with HCV-1 CHC in our randomized trial published recently[5] and found that 150 (75%) of these patients had serum HCV RNA $\geq 10^5$ IU/mL (HVL). The RVR and SVR rates of 150 patients were 64% and 34.7%, respectively, after receiving combination therapy with peginterferon alpha-2a plus oral ribavirin. A significantly higher SVR rate in patients treated for 48 weeks than 24 weeks (78.5%, 62 of 79 versus 35.4%, 34 of 71, $P < 0.001$) was observed. Recently, Ide et al. reported a 36% (20 of 56) SVR rate in Japanese patients with CHC and HVL treated with peginterferon alpha-2b/ribavirin for 48 weeks.[6] Accordingly, we have noticed the SVR rate and RVR rate in Taiwanese patients with HCV-1 with HVL treated for 24 or 48 weeks were higher than Japanese patients treated for 48 or 72 weeks. The important role of the weight-based ribavirin exposure during the first 4 weeks of combination therapy on the achievement of an RVR has been highlighted previously.[7] A cut-off point of 13 mg/kg/day of the first 4 weeks of weight-based ribavirin exposure have been reported associated with the achievement of an RVR.[8] Patients in the study by Shirakawa et al. would have an exposure of less than 13.1 mg/kg/day ribavirin exposure by 4 weeks (starting dose: for body weight 60 kg or less; 600 mg/day, 61 kg to 80 kg; 800 mg/day, 81 kg or more; 1000 mg/day) which is lower than 17.4 mg/kg/day (starting dose: for body weight 75 kg or less; 1000 mg/day, 75 kg or more; 1200 mg/day) in Taiwanese patients infected with HCV-1. We agree that the valuable finding of pretreatment predictors for SVR rate by Shirakawa et al. should be applauded. The relative low SVR and RVR rates, even with longer duration of treatment, compared to Taiwanese patients infected with HCV-1 with HVL raise a concern of relative suboptimal ribavirin exposure, and the actual role of these pretreatment predictors have to be confirmed by further studies.

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Potential conflict of interest: Nothing to report.

Reply:

First, we have to point out a mistake that Dai et al. made in their letter: the rapid virological response (RVR) and sustained virological response (SVR) rates are described inversely with respect to our data and maybe with respect to their data.

Dai et al. reported that the SVR rate of 64% in their study is apparently higher than the rate of 45% in our study. We think that this difference can be attributed to the difference in the mean ages of the patients enrolled in the two studies. The mean age in their study seems to have been around 50 years[1] and was over 10 years lower than that in our study (62 years old). Younger patients tended to show higher SVR rates in our study as well, and the SVR rate in patients who were younger than 55 years old was 61%, which is comparable to the rate reported by Dai et al.

It is possible that a higher administration dose of ribavirin during the first 4 weeks is associated with a higher RVR rate. However, we cannot comment on this because our study was not designed for that purpose.

We adopted the definition of high viral load generally used in Japan (10^5 IU/mL). Since Dai et al. suggested that a difference in the definition of high viral load influenced the results, we recalculated factors associated with SVR in 103 patients whose viral load was higher than 4×10^5 IU/mL and also in 89 patients whose viral load was higher than 8×10^5 IU/mL. The same four prediction factors as those provided by the original analysis of 120 patients whose viral load was higher than 10^5 IU/mL were again selected.

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Distribution of Hepatitis B Virus Genotypes among Patients with Chronic Infection in Japan Shifting toward an Increase of Genotype A▽

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Acute hepatitis B virus (HBV) infection has been increasing through promiscuous sexual contacts, and HBV genotype A (HBV/A) is frequent in patients with acute hepatitis B (AHB) in Japan. To compare the geographic distribution of HBV genotypes in patients with chronic hepatitis B (CHB) in Japan between 2005 and 2006 and between 2000 and 2001, with special attention to changes in the proportion of HBV/A, a cohort study was performed to survey changes in genotypes of CHB patients at 16 hospitals throughout Japan. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses. Of the 1,271 patients, 3.5%, 14.1%, and 82.3% were infected with HBV/A, -B, and -C, respectively. In comparison with our previous survey during 2000 and 2001, HBV/A was twice as frequent (3.5% versus 1.7%; $P = 0.02$). The mean age was lower in the patients with HBV/A than in those with HBV/B or -C. Based on phylogenetic analyses of 11 full-length genomes and 29 pre-S2/S region sequences from patients, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India. They clustered with HBV/A from AHB patients and have spread throughout Japan. HBV/A has been increasing in CHB patients in Japan as a consequence of AHB spreading in the younger generation through promiscuous sexual contacts, aided by a tendency of HBV/A to induce chronic hepatitis. The spread of HBV/A infection in Japan should be prevented by universal vaccination programs.

Hepatitis B virus (HBV), a member of the *Hepadnaviridae*, is a circular, partially double-stranded DNA virus and is one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).

The HBV genome is composed of approximately 3,200 nucleotides. HBV is classified into eight genotypes, designated A to H, based on an intergroup divergence of 8% or more in the complete nucleotide sequence (3, 23, 26, 37). They have distinct geographical distributions and are associated with differences in clinical and virological characteristics, such as severity of liver disease and response to antiviral therapies (7, 8, 12, 13, 22, 28). Furthermore, subgenotypes have been reported for HBV/A, -B, and -C and named A1 to -3 (17, 38), B1 to -6 (31, 32, 40), and C1 to -6 (20, 31, 45). Equally, other genotypes are classified into subgenotypes. There have been increasing lines of evidence to indicate influences of HBV subgenotypes on the outcome of liver disease and the response to antiviral therapies (1, 39, 44).

In 2001, we reported the geographic distribution of HBV genotypes in Japan (27). Of the 720 Japanese patients with chronic HBV infection (CHB), 12 (1.7%) harbored HBV/A, 88 (12.2%) HBV/B, 610 (84.7%) HBV/C, 3 (0.4%) HBV/D, and 7 (1.0%) mixed genotypes. HBV/C was detected in over 94%

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of patients on the Japanese mainland, while HBV/B was found in 64% of those in Okinawa, the southernmost islands, and 44% of those in the Tohoku area in the northern part of the mainland.

Recently, acute HBV infection (AHB) has been increasing in Japan, predominantly through promiscuous sexual contacts. In addition, it was reported that HBV/A was more frequent in patients with acute hepatitis than in those with chronic hepatitis (29, 41, 49). Recent studies suggest that the chances for progression to chronic disease may differ among patients acutely infected with HBV of distinct genotypes (21, 25); patients infected with HBV/A run an increased risk of becoming HBV carriers. Hence, it is of utmost concern whether chronic HBV/A infection is increasing in Japan.

In the present study, we compared the geographic distribution of HBV genotypes in Japan during 2005 and 2006 with 2000 and 2001, with special attention to changes in the proportion of HBV/A. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses.

MATERIALS AND METHODS

Patients. From September 2005 to October 2006, sera were collected from 1,370 consecutive patients with CHB at 16 representative hospitals that were liver centers in their respective regions throughout Japan for the purpose of investigating the geographic distribution of HBV genotypes in Japan. All of the patients were diagnosed after they had been followed for at least 12 months. Patients diagnosed with AHB were excluded from the study; they had a sudden onset of clinical symptoms of hepatitis, along with high-titer antibody to HBV core antigen of the immunoglobulin M class in serum. Their sera were tested for alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and hepatitis B e antigen (HBeAg), as well as antibody to HBeAg (anti-HBe) (Dinabot, Tokyo, Japan). Four clinical diagnoses were established for them. The inactive carrier state was defined by the presence of HBV surface antigen (HBsAg) with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of portal hypertension. Chronic hepatitis was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/liter]) persisting over 6 months (with at least three bimonthly tests). Cirrhosis was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges, and hypersplenism), platelet counts of <100,000/cm^3, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy, or a combination thereof.

The study protocol conformed to the 1975 declaration of Helsinki and was approved by the ethics committees of the respective institutions. Every patient or his/her next of kin gave informed consent to the purpose of the study.

Genotypes and subgenotypes of HBV. The six HBV genotypes (A to F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV Genotype EIA; Institutes of Immunology Co., Ltd., Tokyo, Japan). The method depends on the combination of epitopes on pre-S2 region products detected by monoclonal antibodies that were specific for each of them (46, 47). Subgenotypes of HBV/A, designated A1 and A2, were determined by direct sequencing of the pre-S2/S gene, followed by a phylogenetic analysis.

Quantification of HBV DNA and sequencing. HBV DNA levels in sera were quantitated with a commercial kit (Amplicor HBV Monitor; Roche Diagnostics, Basel, Switzerland) with a detection range from 2.6 to 7.6 log copies/ml. Nucleic acids were extracted from 100 μl of serum using the Qiaamp DNA Blood Minikit (Qiagen GmbH, Hilden, Germany). Eleven complete HBV/A genomes and 29 pre-S2/S region sequences were amplified by PCR with appropriate primer sets, as described previously (40). The amplified HBV DNA fragments were directly sequenced using the ABI Prism Big Dye kit version 3.0 (Applied Biosystems, Foster City, CA) in an ABI 3100 automated DNA sequencer (Applied Biosystems). All sequences were analyzed in both forward and reverse directions. Complete and partial HBV genome sequences were aligned using GENETYX version 11.0 (Software Development Co., Ltd., Tokyo, Japan).

TABLE 1. Characteristics of 1,271 CHB patients

Parameter	Value
Characteristic	
Male gender [no. (%)]	766 (60.3)
Age (yr; mean ± SD)	51.4 ± 14.0
Diagnosis	
Inactive carrier state [no. (%)]	206 (16.2)
Chronic hepatitis [no. (%)]	786 (61.8)
Cirrhosis [no. (%)]	175 (13.8)
HCC [no. (%)]	104 (8.2)
Antiviral treatment [no. (%)]	577 (45.4)
Blood tests	
Platelets (10^4/mm^3)	21.4 ± 30.2
ALT (IU/liter)	59.8 ± 103.0
ALP (IU/liter)	270.4 ± 136.0
γ−GTP (IU/liter)	47.4 ± 66.1
HBV markers	
HBeAg [no. (%)]	399 (31.4)
HBV DNA (median [range] [log copies/ml])	4.2 (<2.6 to >7.6)

Molecular evolutionary analysis of HBV. Reference sequences were retrieved from the DDBJ/EMBL/GenBank databases with their accession numbers for identification. To investigate the relationship between HBV isolates from patients with chronic and acute hepatitis B in Japan, HBV/A isolates (AH1 to -10) were randomly retrieved from them and sequenced in our previous study (29). Nucleotide sequences of HBV DNA were aligned by the program CLUSTAL X, and genetic distance was estimated by the six-parameter method (10) in the Hepatitis Virus Database (36). Based on these values, phylogenetic trees were constructed by the neighbor-joining method (30) with the midpoint rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1,000 times.

Statistical analysis. Categorical variables were compared between groups by the χ^2 test or Fisher's exact test and noncategorical variables by the Mann-Whitney U test. A P value of less than 0.05 was considered significant.

Nucleotide sequence accession numbers. The DDBJ/EMBL/GenBank accession numbers of the complete genome sequences of HBV isolates JPN_CH1 to -11 are AB453979 to AB453989.

RESULTS

Distribution of HBV genotypes among patients with CHB. Of the 1,370 serum samples, the genotype could not be determined for 99 (7.2%) by EIA due to low HBsAg levels, leaving 1,271 for analysis in this study (Table 1). Of these, 206 (16.2%) were inactive carriers, 786 (61.8%) had chronic hepatitis, 175 (13.8%) cirrhosis, and 104 (8.2%) HCC. They had a mean age of 51.4 ± 14.0 years and included 766 (60.3%) men. They had a median HBV DNA level of 4.2 log copies/ml, and 399 (31.4%) of them were positive for HBeAg. Antiviral treatment had been given to 577 (45.4%) of them with interferon, lamivudine, adefovir pivoxil, or entecavir.

The genotypes were HBV/A in 44 (3.5%), HBV/B in 179 (14.1%), HBV/C in 1,046 (82.2%), and HBV/D in 2 (0.2%) (Table 2). In comparison with our previous report on the distribution of genotypes in Japan in 2001 (27), HBV/A was more frequent in this study (3.5% versus 1.7%; $P = 0.02$). Of the 16 hospitals in this study, 10 overlapped with those in our previous report from 2001. In these 10 hospitals, HBV/A was more frequent in the present than in the previous survey (3.6% versus 1.7%; $P = 0.04$).

The distribution of HBV genotypes in Japan differed by

TABLE 2. Distribution of HBV Genotypes

Genotype	No. (%)	
	2005–2006 (n = 1,271)	2000–2001[a] (n = 720)
A	44 (3.5[b])	12 (1.7)
B	179 (14.1)	88 (12.2)
C	1,046 (82.3)	610 (84.7)
D	2 (0.2)	3 (0.4)
Mixed	0 (0.0)	7 (1.0)

[a] From Orito et al. (27).
[b] P = 0.02.

geographic location (Fig. 1). HBV/C was the most prevalent in the majority of areas. In the Tohoku area, the northern part of the Japanese mainland (Honshu), HBV/B was more prevalent than in the other areas of the Japanese mainland. In Okinawa, the southernmost islands of Japan, HBV/B was predominant. Of note, HBV/A was more frequent in the Kanto area (9.5%), the metropolitan area, and Okinawa (9.1%) than in the other areas.

Clinical differences among HBV/A, -B, and -C. Clinical backgrounds were compared among the patients infected with HBV/A, -B, and -C (Table 3). HBeAg was significantly less prevalent in the patients infected with HBV/B than in those infected with HBV/A or -C ($P < 0.01$ for each). When the positivity of HBeAg was stratified by age, HBeAg was markedly less common in patients infected with HBV/B than in those infected with HBV/A or -C who were older than 40 years of age (7/157 [4.5%] versus 4/19 [21.1%] [$P < 0.05$] or 215/755 [28.5%] [$P < 0.01$]) (Fig. 2). There were no significant differences in HBV DNA levels among patients infected with the three genotypes. As antiviral treatments might have influenced the severity of liver disease, clinical states were compared among patients infected with HBV/A, -B, and -C who did and did not receive it; antiviral treatments did not affect the above-mentioned trends represented in Table 3 in age, diagnosis, and HBeAg, as well as ALT and HBV DNA levels (data not shown).

Additionally, we compared the distributions of age and liver diseases in patients infected with HBV/A, -B, and -C. In patients infected with HBV/C, the prevalence of cirrhosis and HCC increased in those older than 50 years of age compared to younger patients (Fig. 3), whereas in the patients infected with HBV/B, cirrhosis and HCC were rare in elderly patients. The proportion of patients younger than 40 years of age was higher in those infected with HBV/A than in those infected with HBV/B or -C (25/44 [56.8%] versus 22/179 [12.3%] or 288/1,046 [27.5%]; $P < 0.01$ for each), while cirrhosis and HCC were also found in those older than 50 years of age infected with HBV/A.

Coinfection with human immunodeficiency virus type 1 (HIV-1) was found in 6 of the 44 (13.6%) patients infected with HBV/A compared to only 3 of the 1,046 (0.3%) patients infected with HBV/C ($P < 0.0001$); it occurred in none of the 179 patients infected with HBV/B.

Phylogenetic analyses. Among the 44 HBV/A isolates, the complete genome was sequenced successfully in 11 (JPN_CH1 to -11). Seven of them were classified as HBV/A2 and four as HBV/A1. A phylogenetic tree was constructed based on the complete genome sequences of these 11 isolates, along with those from two patients with AHB and those from 40 HBV/A isolates retrieved from the database (Fig. 4). Of the seven HBV/A2 isolates, the four from patients with CHB in this study formed a cluster with the Japanese isolates retrieved from the database and two from patients with AHB. Of the other three isolates, JPN_CH5 clustered with French and U.S. isolates, JPN_CH6 with German isolates, and JPN_CH7 with

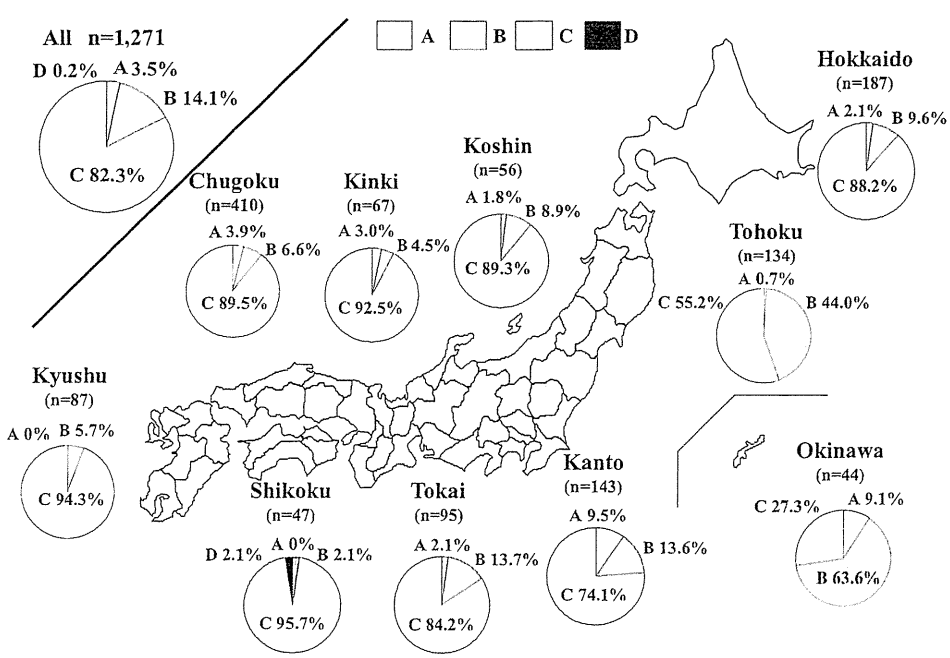

FIG. 1. Geographic distribution of HBV genotypes in patients with chronic HBV infection in Japan during 2005 and 2006.

TABLE 3. Clinical characteristics of individuals chronically infected with HBV of different genotypes

Parameter	Value for genotype:		
	A (n = 44)	B (n = 179)	C (n = 1,046)
Male gender [no. (%)]	32 (72.7)	112 (62.6)	621 (59.4)
Age (yr [mean ± SD])	41.3 ± 14.9[a]	55.8 ± 13.7[b]	48.8 ± 13.3
Diagnosis			
Inactive carrier state [no. (%)]	13 (29.5)[c]	63 (35.2)[b]	129 (12.3)
Chronic hepatitis [no. (%)]	26 (59)	103 (57.5)	656 (62.7)
Cirrhosis [no. (%)]	3 (6.8)	10 (5.6)[b]	162 (15.5)
HCC [no. (%)]	2 (4.5)	3 (1.7)[b]	99 (9.5)
Anti viral treatment [no. (%)]	13 (29.5)[d]	48 (26.8)[b]	516 (49.3)
Blood tests			
Platelet (10^4/mm^3)	23.3 ± 21.9	25.9 ± 35.9[e]	20.6 ± 29.5
ALT (IU/liter)	56.2 ± 83.8	42.2 ± 104.2[e]	63.0 ± 103.3
ALP (U/liter)	247.1 ± 123.0	255.5 ± 97.9	273.9 ± 141.9
γ-GTP (U/liter)	39.6 ± 34.6	49.3 ± 63.4	47.5 ± 67.6
HBV markers			
HBeAg [positive rate(%)]	15 (34.0)[f]	17 (9.5)[b]	367 (35.1)
HBV DNA (median [range]) (log copies/ml)	4.2 (<2.6–>7.6)	4.1 (<2.6–>7.6)	4.2 (<2.6–>7.6)

[a] $P < 0.01$, A versus B or C.
[b] $P < 0.01$, B versus C.
[c] $P < 0.01$, A versus C.
[d] $P < 0.05$, A versus C.
[e] $P < 0.05$, B versusC.
[f] $P < 0.01$, A versus B.

Spanish and Italian isolates. All four HBV/A1 isolates in this study formed a cluster with Philippine and Indian isolates.

In addition, the pre-S2/S region sequences of a total of 29 isolates were determined, including the 11 isolates whose complete genomes were sequenced. Of these, 21 (72%) were classified as HBV/A2 and the remaining 8 as HBV/A1. A phylogenetic tree was constructed based on the pre-S2/S region sequences from the 29 isolates, along with those from 10 patients with AHB infected with HBV/A and 47 HBV/A isolates retrieved from the database (Fig. 5). The 21 HBV/A2 isolates in the present study formed a cluster with Japanese, American, and European isolates retrieved from the database and those from patients with acute hepatitis. In addition, some of them were highly homologous with each other. Likewise, HBV/A1 isolates from eight patients with chronic hepatitis in this study were highly homologous with those from two patients with acute hepatitis and isolates from the Philippines and India. Based on the phylogenetic analyses, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India, and had infiltrated throughout Japan.

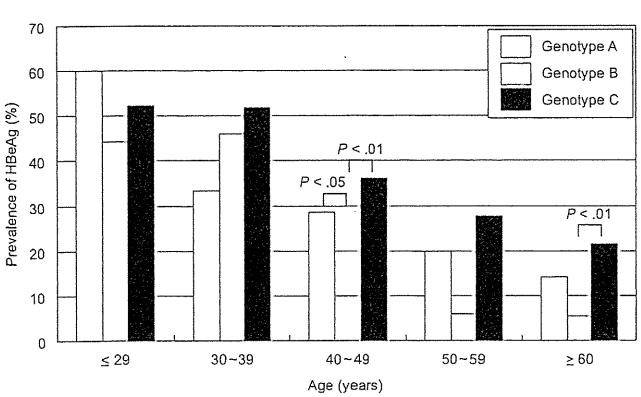

FIG. 2. Prevalence of HBeAg among patients infected with HBV of different genotypes stratified by the age.

DISCUSSION

Perinatal transmission from carrier mothers to their babies has been the principal route for establishing persistent HBV infection in Asian countries (19). In Japan, passive and active immunoprophylaxis with HBV immune globulin and vaccine has been mandated for babies born to HBeAg-positive carrier mothers since 1986; this was extended to HBeAg-negative carrier mothers in 1995. As a result, HBsAg has become rare in Japanese born after 1986; it was detected in only 0.2% of first-time blood donors younger than 19 years of age in 2000 (24). However, AHB has been increasing in Japan, predominantly through promiscuous sexual contacts.

In Japan, HBV/A is detected rarely among patients with CHB but is frequent in those with acute hepatitis (14, 25, 29, 41, 43). Yotsuyanagi et al. reported the distribution of genotypes in 145 Japanese patients with AHB and found HBV/A in 27 (19%), HBV/B in 8 (5%), and HBV/C in 109 (75%) (49). HBV/A is more frequent in metropolitan areas than other areas. The majority of patients with HBV/A infection in metropolitan areas have had extramarital sexual contacts with multiple irregular partners, through which they could have contracted infection. In support of this view, among men who have sex with men (MSM) who are coinfected with HBV and HIV-1 in Tokyo, most were infected with HBV/A (15, 35).

In Japan, AHB in adulthood becomes chronic in only ~1%

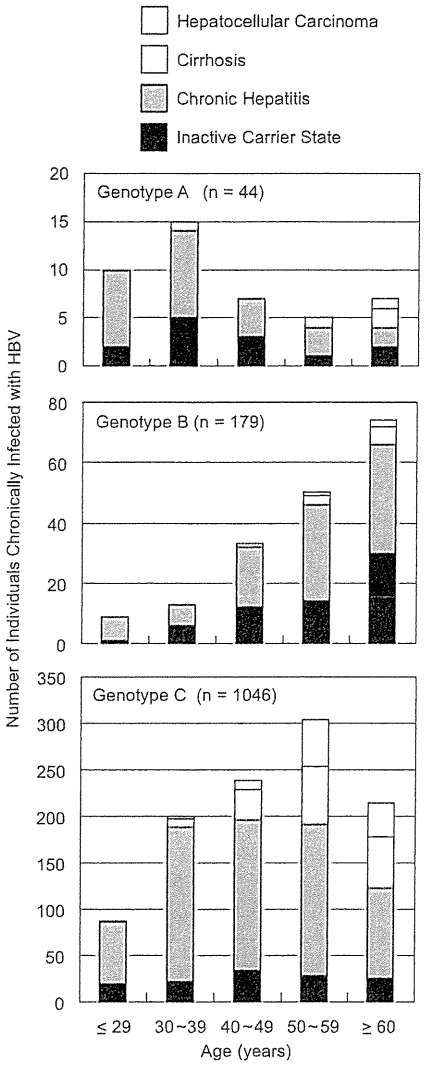

FIG. 3. Distribution of HCC, cirrhosis, chronic hepatitis, and inactive carrier state among the 1,271 patients infected with HBV of different genotypes stratified by the age.

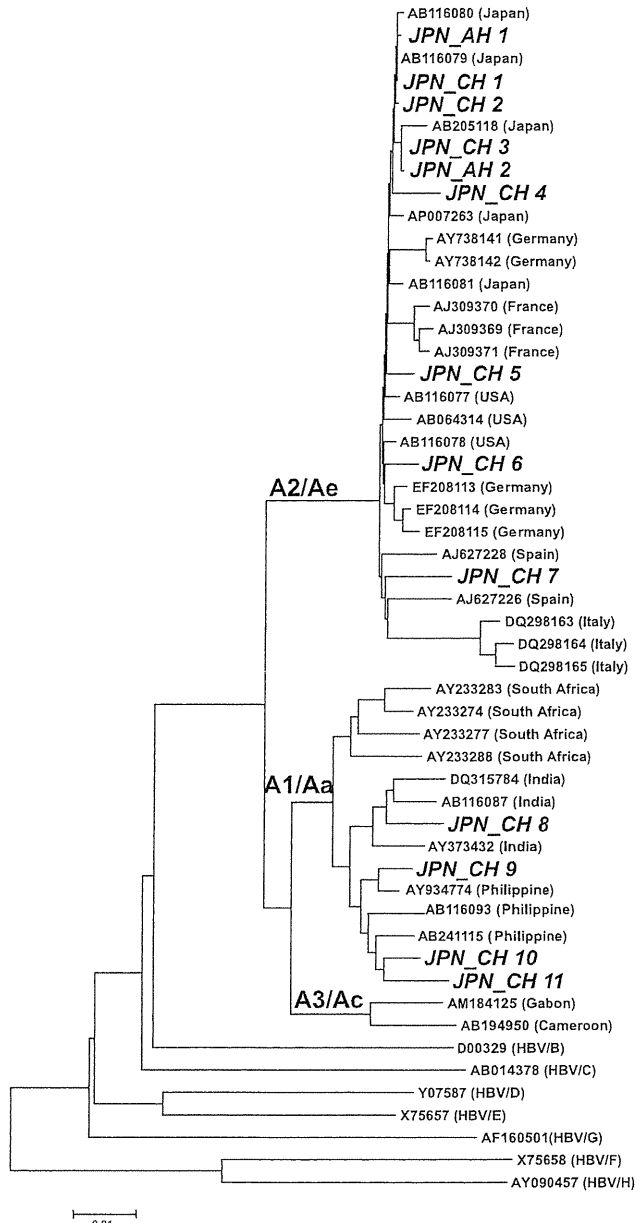

FIG. 4. Phylogenetic tree constructed based on the complete genome sequences of HBV/A isolates. Those from 11 patients with chronic infection in this study are shown in boldface italic (JPN_CH1 to -11), along with two isolates (JPN_AH1 and -2) from patients with acute hepatitis in Japan reported in our previous study (17). Representative isolates were retrieved from the DDBJ/EMBL/GenBank databases, including 21 HBV/Ae, 10 HBV/Aa, and 2 HBV/Ac isolates, along with 7 HBV isolates representative of the other seven genotypes. Isolates from the databases are identified by accession numbers, followed by the country of origin. The bar at the bottom spans 0.01 nucleotide substitutions per site.

of cases. This is much less than the progression to chronic disease (close to 10%) in Europe and the United States, where HBV/A prevails (34). Recent studies have suggested that the chances for persistence may differ among patients acutely infected with HBV of distinct genotypes (21, 25). In particular, acute infection with HBV/A may bring about an increased risk of progression to chronic disease. Therefore, an increase of acute infection with HBV/A would result in a surge of HBV/A among patients with CHB in Japan. In actuality, in comparison with our previous results during 2000 and 2001 (27), HBV/A was twice as frequent in this study (3.5% versus 1.7%; $P = 0.02$). HBV/A has been increasing in patients with CHB in the Kanto area, where HBV/A in patients with acute hepatitis is more frequent than in the other areas. In the islands of Okinawa, also, HBV/A was found to be prevalent in this study. Of the four patients infected with HBV/A there, two were coinfected with HIV-1. They were both MSM, and they were suspected to have been infected with HIV through sexual contacts on the Japanese mainland. It has been reported that HIV infection increases the probability that AHBs will become chronic (2, 11, 33, 48). Because they share routes of transmission and the risk for HIV-1 and HBV infections, approximately

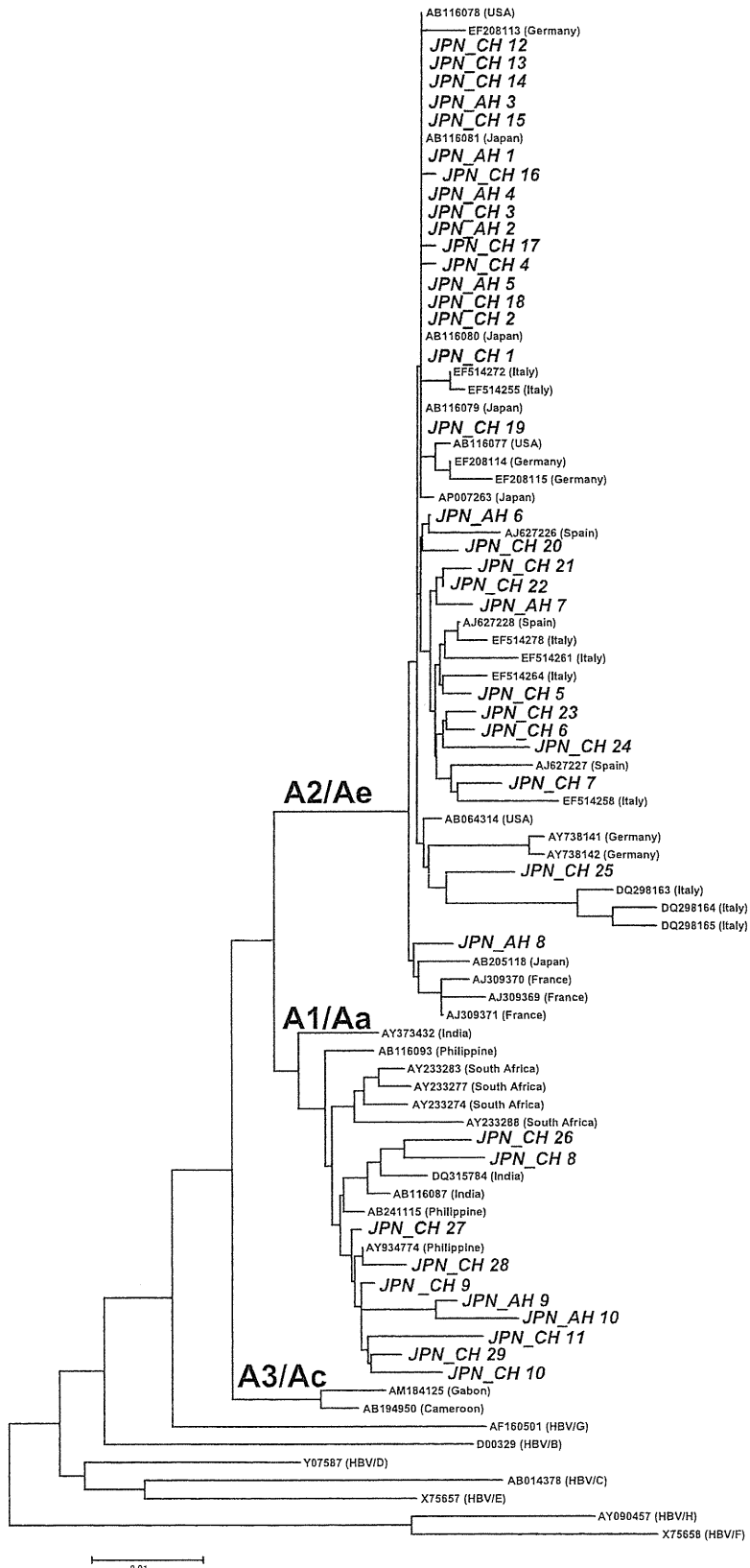

FIG. 5. Phylogenetic tree constructed based on pre-S2/S region sequences of HBV/A isolates. Those from 29 patients with chronic infection in this study are shown in boldface italic (JPN_CH1 to -29), along with 10 isolates (JPN_AH1 to -10) from patients with acute hepatitis in Japan reported in our previous study (17). Representative isolates were retrieved from the DDBJ/EMBL/GenBank databases, including 28 HBV/Ae, 10 HBV/Aa, and 2 HBV/Ac isolates and 7 HBV isolates representative of the other seven genotypes. Isolates from the databases are identified by accession numbers, followed by the country of origin. The bar at the bottom spans 0.01 nucleotide substitutions per site.

90% of patients with AIDS have markers of past or ongoing HBV infection (18). Thus, HBV carriers are more frequent in the HIV-1-positive than in the HIV-1-negative population (4, 9). Among patients with HIV infection in Japan, 6.3% are HBsAg positive, in particular, 8.3% of HIV-infected MSM (16). In this study, coinfection with HIV was found in 6 of the 44 (13.6%) patients infected with HBV/A. All of them were men. Their median age was 27.7 ± 4.1 years, and five patients were positive for HBeAg. Thus, there is a possibility that HIV-1 and HBV/A coinfections are increasing among young people in Japan, and the high rate of HBeAg positivity may be influenced by immune suppression due to HIV infection.

In the phylogenetic analysis, the HBV/A2 isolates recovered in this study were homologous to those from Europe and the United States, and some of them clustered with the Japanese isolates. On the other hand, there were HBV/A1 isolates that formed a cluster with those from the Philippines and India. Furthermore, some isolates from patients with acute hepatitis who were infected with HBV/A in Japan were highly homologous to HBV/A isolates from patients with chronic hepatitis. This invites speculation that some HBV/A isolates were introduced into Japan from foreign countries, while others have already settled down there and spread from patients with chronic infection to their contacts. HBV/A would have been infiltrating throughout Japan by these two different routes.

Clinical differences among patients infected with HBV/A, -B, and -C were observed. The mean age was lower in the patients infected with HBV/A than in those infected with HBV/B or -C. As mentioned above, AHB patients infected with HBV/A have been increasing in the younger generation in Japan, and around 10% of them would have progressed to chronic infection. This is one of the reasons why the patients infected with HBV/A are younger than those infected with HBV/B or -C. Most patients infected with HBV/B were negative for HBeAg, while a high proportion of the patients infected with HBV/A and -C had it. In particular, this difference was remarkable in the patients who were older than 40 years of age. Thus, the seroconversion rate for the loss of HBeAg among younger people may be higher in infection with HBV/B than in that with HBV/A or -C. Inactive carriers were commoner in HBV/A than in HBV/C infection, as well.

These lines of evidence indicate that the activity of hepatitis is lower in HBV/B than HBV/C infection, and patients with HBV/B seroconvert from HBeAg to anti-HBe at young ages. In addition, cirrhosis and HCC were less frequent in the patients infected with HBV/B than in those infected with HBV/C. Therefore, the prognosis would be better in the patients infected with HBV/B than in those infected with HBV/C. These results are in accord with previous reports (5, 13, 28, 42). There have been few reports on the clinical features of patients with chronic hepatitis infected with HBV/A in Japan. Chu et al. have reported the distribution of HBV genotypes with reference to clinical characteristics in the United States (6). They have shown that HBV/A and HBV/C infections are accompanied by a higher frequency of HBeAg than HBV/B infection, while HBV/B is associated with a lower rate of hepatic decompensation than HBV/A and -C. In our study, inactive carriers were commoner, while cirrhosis and HCC were found less often in HBV/A than in HBV/C infection. HBeAg was more prevalent in the patients infected with HBV/A than in those infected with HBV/B who were older than 40 years of age. Therefore, it can be said that the prognosis is better for patients infected with HBV/A than for those infected with HBV/C; it may be poorer than for those infected with HBV/B.

In conclusion, HBV/A has been increasing among CHB patients in Japan. On the basis of phylogenetic analyses, some HBV/A isolates appear to have been imported from foreign countries. They clustered with HBV/A from AHB patients and have infiltrated throughout Japan. It is very likely that acute and chronic infections with HBV/A have been increasing in Japan. Obviously, immunoprophylaxis of perinatal HBV infection, implemented since 1986 on a national basis, has been insufficient to prevent horizontal HBV/A infection diffusing among high-risk groups by transmission routes shared by HIV infection. The foreseeable spread of HBV/A infection in Japan should be prevented by universal vaccination programs extended to high-risk groups or the general population.

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Epidemiology of hepatocellular carcinoma in Japan

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Primary liver cancer, 95% of which is hepatocellular carcinoma (HCC), is ranked third in men and fifth in women as a cause of death from malignant neoplasms in Japan. The number of deaths and death rate of HCC began to increase sharply in 1975. These numbers peaked at 34510 and 27.4/100000, respectively, in 2004, but decreased to 33662 annual deaths and a 26.7/100000 death rate in 2006. Although hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are both major causes of HCC, HCV-related HCC represents 70% of all cases. The incidence of HCC without hepatitis B surface antigen (HBsAg) or antibodies to HCV (anti-HCV) accounts for 8%–15% of HCC patients nationwide. Geographically, HCC is more frequent in western than eastern Japan, and death rates of HCC in each prefecture correlate with anti-HCV, but not HBsAg, prevalence. Interferon therapy for chronic hepatitis C reduces the risk of development of HCC, especially among patients with sustained virological response. Further research should focus on the mechanisms of carcinogenesis by HCV and HBV, development of more effective treatments, and establishment of early detection and preventative approaches. Better understanding of HCC unrelated to HCV and HBV, possibly caused by steatohepatitis and diabetes, should also be a major concern in future studies.

Key words: HCC, HCV, HBV, nonalcoholic steatohepatitis (NASH), interferon

Introduction

The three leading causes of death in Japan since 1981 are malignant neoplasms, cardiovascular diseases, and cerebrovascular diseases. For the past 30 years, liver cancer has been the third leading cause of death from malignant neoplasms in men, following lung and stomach cancer. In women, liver cancer has ranked fifth as a cause of death during the past decade, following colon, stomach, lung, and breast cancer. Primary liver cancer can be classified into three types according to the cell from which the cancer originated, namely, hepatocellular carcinoma (HCC), cholangiocellular carcinoma, and other. As HCC accounts for up 95% of all primary cancer cases, the term "liver cancer" usually means HCC.[1]

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the two major causes of HCC in Japan.[2,3] The increase in incidence of HCC in Japan, however, is largely attributable to the increase of HCV infection in the general population during the past 50 to 60 years.[2]

Changes in deaths and death rates of primary liver cancer

Changes in annual deaths from primary liver cancer among different age groups between 1958 and 2006 are shown in Fig. 1. The total number of deaths from HCC was stable at fewer than 10000 persons/year until 1975 before showing a sharp increase. The spike in 1995 resulted from a change in the International Classification of Disease (ICD) code from ICD 9 to ICD 10, which included intrahepatic bile duct cancer, accounting for approximately 5% of HCC deaths.

The majority of HCC mortalities were in patients below the age of 69 until 1999, when this age reached 70 years. In 2006, 66% of patients with HCC were over 70. The number of deaths from HCC reached 34510 in 2004, but decreased to 33662 in 2006.

The death rates of liver cancer by sex (Fig. 2) are consistently higher in men than in women. A sharp rise in the death rate of primary liver cancer in men began

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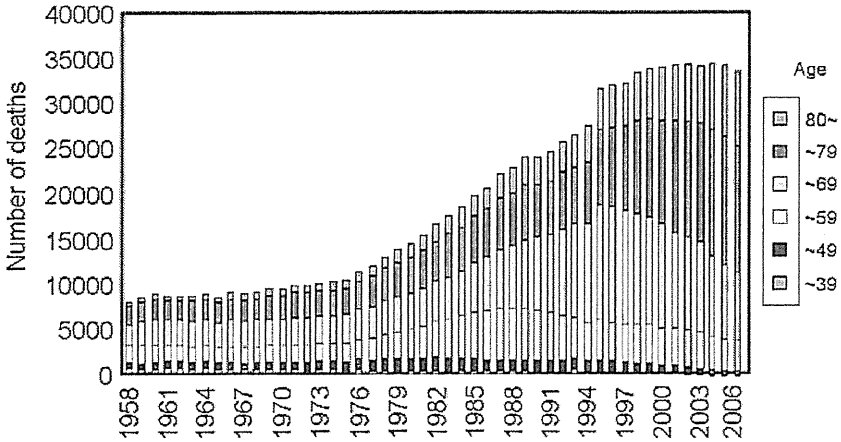

Fig. 1. Changes in annual deaths of patients (by age, in years) with primary liver cancer between 1958 and 2006. (Taken from the Vital Statistics of Japan, released every year by the Ministry of Health, Labour, and Welfare)

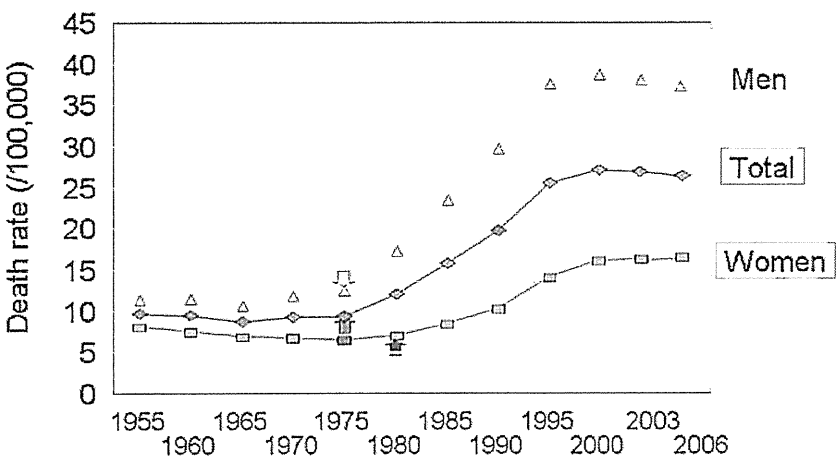

Fig. 2. Changes in the death rate of primary liver cancer in men (*triangles, yellow*), women (*rectangles, pink*), and in total (*diamonds, blue*)

in 1975, and a more gradual rise in women commenced in 1980. The total age-adjusted death rate peaked in 2002 (27.5/100 000 persons in 2002), and decreased to 27.0 in 2003. In 2006, the total age-adjusted death rate stood at 26.7/100 000, which is caused by a decrease in death rate (36.7) in men, but offset by an increase in women to 17.2.

Age and sex in HCC

Changes in the mean age of HCC patients and male/female ratio every 2 years between 1984 and 2003 are shown in Fig. 3. In that period, the mean age of female HCC patients was higher than that of males, and the mean ages of both sexes progressively increased. As reported previously, however, HBV-related HCC was stable from 1982 to 2003, implying that this change originated from HCV-related HCC patients. The male/female ratio was 4.5 in 1984–1985 and 2.5 in 2002–2003 (see Fig. 3), showing that the proportion of female patients with HCC had increased. This increase in female patients is also considered as a consequence of increased HCV-related HCC.

Changes in etiology of HCC in Japan

A nationwide survey on primary liver cancer has been conducted every 2 years since 1968 by the Liver Cancer Study Group of Japan.[1,4–9] Five serological surveys performed between 1990 and 2001 have documented that most patients with HCC are positive for either HBsAg or antibodies to HCV (anti-HCV). Tests for HBsAg became available in 1975 and those for anti-HCV in 1990. HBsAg-positive cases of HCC constituted 42% of patients in 1977–1978, but only 15.5% in 2002–2003 (Fig. 4). In contrast, anti-HCV-positive cases of HCC accounted for more than 70% of cases diagnosed until 2000–2001. However, this number dipped to 69.6% in 2002–2003, and has since remained at less than 70%. In contrast, HCV of unknown origin and other cases of HCC have been increasing gradually, and constituted 14.9% of all cases in 2002–2003.

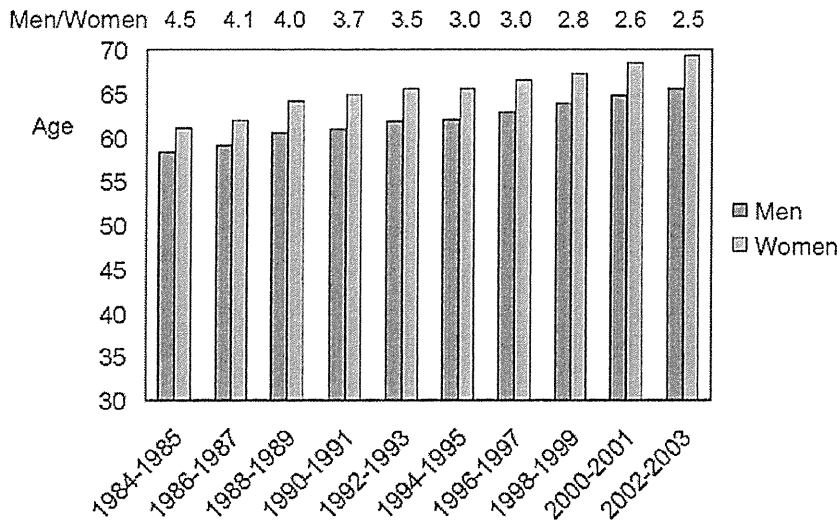

Fig. 3. Changes in the mean age (in years) of men (*blue bars*) and women (*pink bars*) patients with hepatocellular carcinoma (HCC) between 1984 and 2003

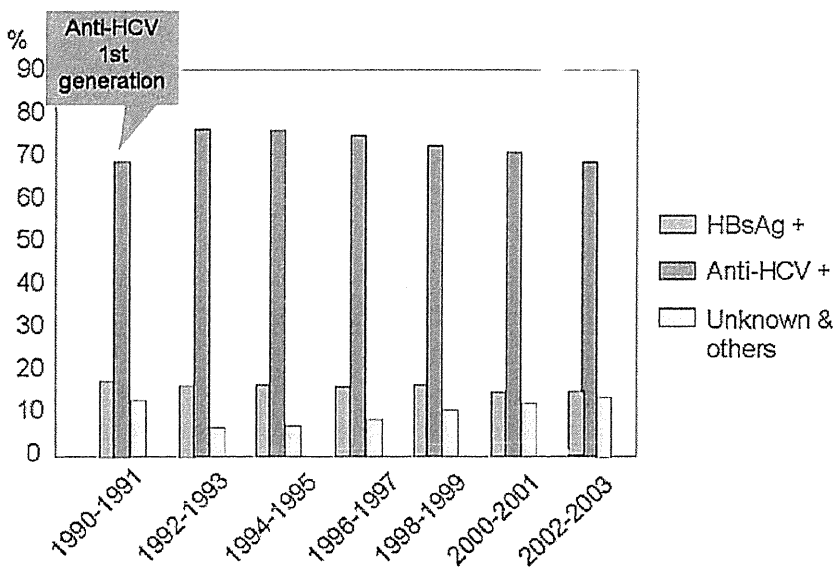

Fig. 4. Changes in the etiology of HCC between 1990 and 2003: hepatitis B surface antigen (*HBsAg+, pink*), antihepatitis C virus (*anti-HCV+, blue*), and *unknown* and *others* (*green*)

In cross-sectional studies conducted at Shinshu University Hospital, HCV-related HCC was found in the majority of cases (72%) (Fig. 5). Non-B non-C HCC (NBNC-HCC) accounted for 10% of cases in 2002–2007. In these 28 patients, nonalcoholic steatohepatitis (NASH) accounted for 14%.

Geographic variation of liver cancer and HBV/HCV infection

Although Japan is a relatively small country with a homogeneous population, the incidence of HCC varies greatly among different regions. The Vital Statistics of Japan for 2005 published in 2007 by the Japanese Ministry of Health, Labour, and Welfare on the incidence of deaths as a result of HCC in its 48 prefectures shows a steady increase in death rates of HCC from east to west in Japan. The average age-adjusted death rate of HCC among 48 prefectures was 27.2 per 100000 persons in 2005 (Fig. 6). Furthermore, nationwide health screening for HBsAg and anti-HCV in citizens over 40 years of age has been performed since 2002, and the prevalence rates of these markers have been analyzed for each prefecture in Japan. In 2006, the average HBsAg and anti-HCV prevalences were 1.0% and 0.7%, respectively, in this group (see Fig. 6). There was a highly significant association between the death rate of HCC and prevalence of anti-HCV in each prefecture (Fig. 7; correlation coefficient = 0.66; $P < 0.001$,

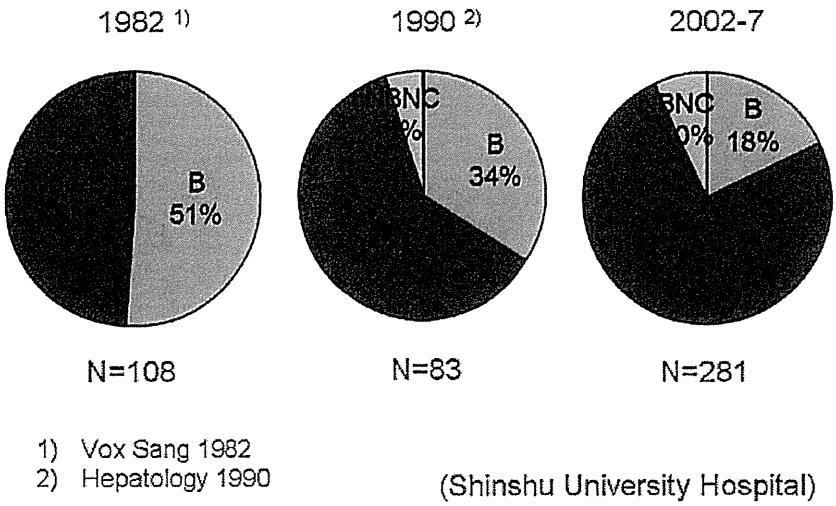

Fig. 5. Clinical features of hepatitis B (*B*) virus (HBV)- and hepatitis C (*C*) virus (HCV)-related HCC in 1982, 1990, and 2002–2005 at Shinshu University Hospital. *NBNC*, non-B non-C

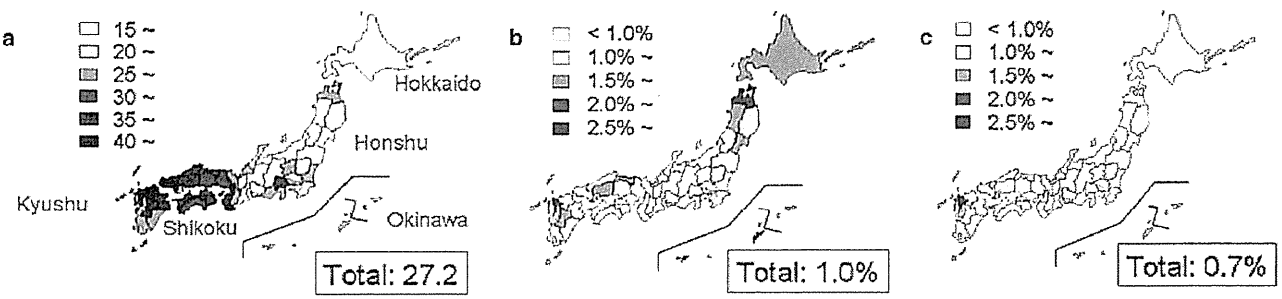

Fig. 6. **a** Death rate of primary liver cancer was 27.2 per 100000 in 2005 among people over 40 years of age in 48 prefectures in Japan. In the same group in 2006, HBsAg prevalence was 1.0% (**b**) and anti-HCV prevalence was 0.7% (**c**)

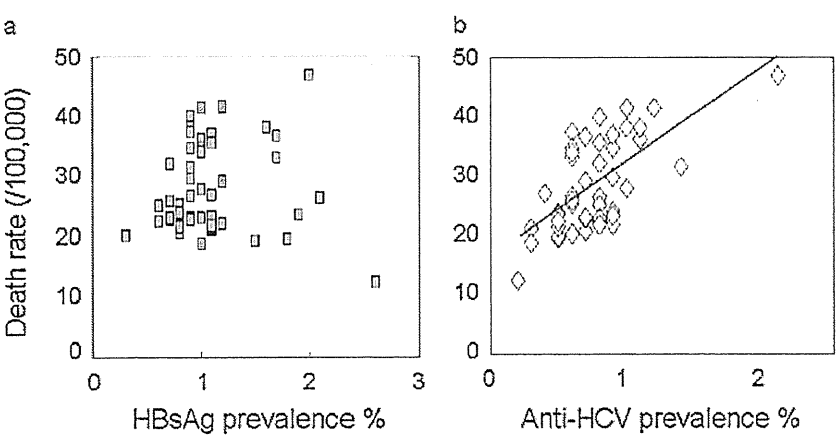

Fig. 7. Relationship between the death rate of primary liver cancer and prevalence of (**a**) HBsAg ($r = 0.02$, P = NS) and (**b**) anti-HCV ($r = 0.66$, $P < 0.001$, $y = 16.3x + 16.1$) among the general population over 40 years of age in 2006

$y = 16.3x + 16.1$), but no correlation with the prevalence of HBsAg was seen (Fig. 6). For instance, although Okinawa Prefecture had the highest prevalence of HBsAg (2.6%), its HCC death rate was the lowest (12.5/100000 persons). A possible explanation for this discrepancy is that the HBV genotype Bj, which shows good clinical prognosis,[10,11] is the dominant HBV genotype in Okinawa. In contrast, areas with high rates of anti-HCV, especially in western Japan, had high death rates from HCC. HCV appears to be the major contributor to primary liver cancer in these regions; Saga Prefecture shows both the highest HCC death rate (46.9/100000) and highest prevalence rate of anti-HCV (2.1%) in Japan.

Table 1. Summary of findings in representative studies on the incidence of hepatocellular carcinoma (HCC) among patients with chronic hepatitis C virus (HCV) infection treated with interferon alone in Japan

	Untreated		Treated					
			Non-SVR		SVR		Total	
Author	No. HCC/no. cases	%	No. HCC/no. cases	%	No. HCC/no. cases	%	No. HCC/no. cases	%
Kasahara[12]			41/709	5.8	5/313	1.6	46/1022	4.5
Imai[13]	19/140	13					18/419	4.3
Ikeda[14]	67/452	15	23/730	3.2	5/461	1.1	28/1191	2.4
Yoshida[15]	67/395	17	214/1556	13.8	27/836	3.2	241/2392	10.1
Okanoue[16]			119/849	14.0	8/397	2.0	127/1246	10.2
Ikeda[17]	59/352	17	34/171	19.9	1/53	1.9	94/576	16.3
Total	212/1339	16	432/4015	10.8	46/2060	2.2	554/6846	8.1

SVR: sustained virological response

Antiviral therapy suppresses the incidence of HCC

As described in prior sections, HCV infection is the major cause of HCC in Japan, suggesting that eradication of HCV may decrease the incidence of HCC. A summary of different studies on the incidence of HCC among patients with chronic hepatitis C who were treated with interferon in Japan can be found in Table 1.[12–17] These studies show a moderate decrease in the risk of HCC in patients with chronic hepatitis C treated with interferon, especially in patients with sustained virological response as compared with nonresponders and nontreated patients.

Recently, Ikeda et al. prospectively studied patients with chronic HCV infection and evidence of occult HBV infection [negative results for HBsAg and HBV DNA but positive results for antibodies to hepatitis B core antigen (anti-HBc) in serological testing].[17] Patients with HCV-related cirrhosis and positive results for anti-HBc were at high risk for HCC, even in patients with a sustained virological response to interferon (IFN) therapy. Thus, anti-HBc positivity is a marker of high risk for HCC among patients with HCV-related cirrhosis.

Between 1992 and 2001, approximately 300000 patients with chronic hepatitis C received IFN monotherapy in Japan. As shown in Fig. 1, it is remarkable that the number of deaths and the death rate of HCC began to decrease in 2005. These phenomena suggest that antiviral treatment indeed reduces the risk of HCC in patients with HCV infection.

Conclusion

The number of deaths and death rate of HCC showed a sharp increase from 1975 onward but had begun to decrease in 2006. Although both HBV and HCV infection play a major role in HCC in Japan, HCV-related HCC represents 70% of all cases. The incidence of HCC without HBsAg or anti-HCV accounts for 7%–15% in Japan, and half of NBNC-HCC cases are of unknown origin. Geographically, HCC is more frequent in western than eastern Japan, and the death rates of HCC in each prefecture correlate with anti-HCV, but not HBsAg, prevalence. IFN therapy for chronic hepatitis C reduces the risk of development of HCC, especially in patients with sustained viral response.

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