Table 3 Percentage of detectable linearized HBsAg and HBcrAg after HBsAg seroclearance

Time from HBsAg seroclearance (months)	Linearized HBsAg		HBcrAg	
	Detectable percentage (ratio)	*p* value	Detectable percentage (ratio)	*p* value
a: 12 months				
≤12	29.1% (52/179)	0.146	24.6% (44/179)	0.079
>12	28.2% (33/150)		20.0% (25/125)	
b: 24 months				
≤24	28.4% (71/250)	0.076	20.0% (50/250)	0.441
>24	17.7% (14/79)		24.1% (19/79)	
c: 36 months				
≤36	25.8% (73/283)	0.901	20.1% (57/283)	0.358
>36	26.1% (12/46)		26.1% (12/46)	

Patients were divided into two groups for analyses based on the time of measurement after HBsAg seroclearance as documented by a conventional HBsAg assay: 12 months (a), 24 months (b), and 36 months (c)

HBsAg hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

is reported to be between 13.5 and 17% [21–23], it may be difficult to differentiate CHB patients with HBsAg seroclearance and those with prior exposure to HBV, especially for persons older than 50 years (the median age of HBsAg loss). Although serum HBV DNA testing is an option for differentiation, the likelihood of a positive HBV DNA result is low as shown in our study (2.1%). Even when the lower limit of detection was improved to 1.1 IU/mL, the chance of HBV DNA positivity was still only 13.4% [9]. The HBcrAg and linearized HBsAg assays would be a useful tool in diagnosing CHB patients with prior HBsAg seroclearance documented by conventional HBsAg assay before the initial clinical presentation, especially since our present study showed the two viral proteins could be detected up to 118 and 187 months after HBsAg seroclearance.

Our present study found that the percentage of detectable serologic markers remained similar in serum samples taken at different time points after HBsAg loss. Moreover, the median levels of both viral proteins among patients with detectable markers after HBsAg seroclearance remained similar over time. Our results suggest that there is persistent low-grade viral transcriptional activity for years after HBsAg seroclearance. In addition, the concept of HBsAg seroclearance might need refinement, given that 25.8% patients with documented HBsAg seroclearance using a conventional assay had a positive linearized HBsAg result. However, it should be noted that these findings are limited by the cross-sectional nature of our study, with the serum of patients taken at different time intervals after HBsAg seroclearance. Longitudinal studies with serial viral protein levels are needed and would be useful to further investigate the relationship of anti-HBs with linearized HBsAg.

The pathophysiology of continued viral protein production after HBsAg seroclearance is not well-defined. The presence of viral escape mutants in the "a" determinant is possible [24]. Although the majority of mutants are detectable by current serologic assays, detection limits of HBsAg mutants vary between different assays [25]. HBsAg production exceeds the required amount for virion assembly of the Dane particle, and can also be secreted as empty subviral particles [26, 27]. These subviral particles have been suggested to be involved in the immune evasion strategy of HBV [28], and could remain in circulation even when the production of virions decreases [29], as in HBsAg seroclearance. In CHB patients achieving HBsAg seroclearance, intrahepatic cccDNA is still detectable at extremely low levels [9]. Serum HBcrAg levels had been previously proven to have good correlation with intrahepatic cccDNA levels [16]. Despite earlier evidence of good correlation between serum HBsAg and intrahepatic cccDNA [30, 31], a recent study did not find such a correlation in HBeAg-negative disease [32], probably since viral integration, a non-essential event in the life cycle of HBV, produces HBsAg in the absence of viral replication [33]. Hence, it is not surprising that studies have shown that there is no significant HBsAg decline in CHB patients treated with nucleoside analogues [34]. Further studies are needed to determine if any correlation exists between linearized HBsAg levels and intrahepatic cccDNA.

Identifying patients with prior HBsAg seroclearance as documented by a conventional assay carries several clinical implications. First, an older age at HBsAg seroclearance is still associated with risk of HCC [9, 10], thus detecting past HBsAg seroclearance would facilitate the enlisting of such patients into HCC surveillance programs. Second, fulminant HBV reactivation is possible in HBsAg-negative but anti-HBc positive patients undergoing immunosuppression or chemotherapy, especially for regimens containing rituximab [35]. Identifying patients with prior HBsAg

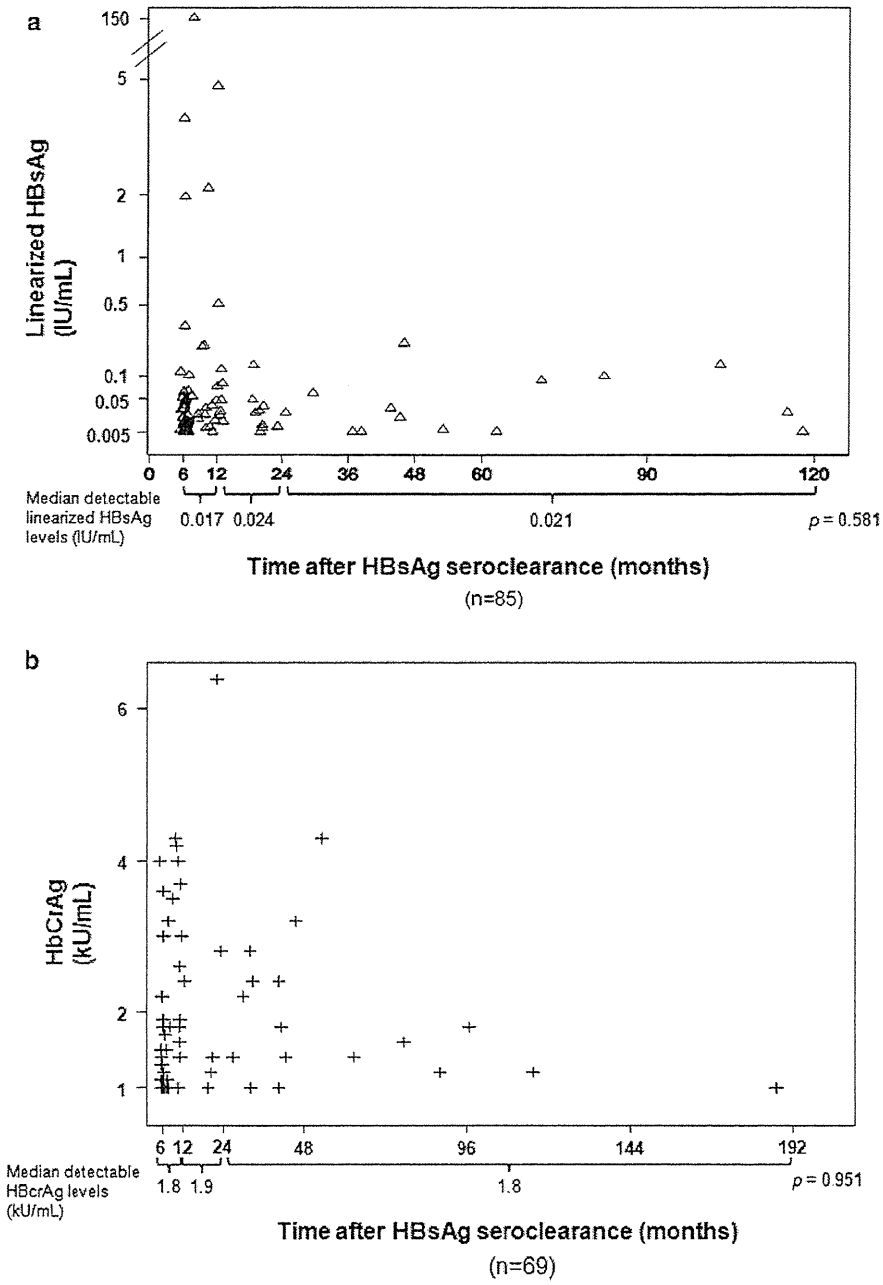

Fig. 3 Detectable linearized HBsAg (**a**) and HBcrAg (**b**) levels after HBsAg seroclearance as documented by a conventional HBsAg assay

seroclearance would allow either preemptive antiviral therapy or close serologic and virologic monitoring. Third, using both HBcrAg and linearized HBsAg assays, over 40% of CHB patients with prior HBsAg seroclearance could be identified. Detectable HBcrAg and linearized HBsAg in patients considered to have cryptogenic cirrhosis will reveal the actual diagnosis to be occult hepatitis B infection. Future studies could further investigate the relationship of these two novel serologic markers with both serum anti-HBs and anti-HBc.

There are several limitations of this study, the first being its cross-sectional nature as mentioned above. HBV genotypes were not checked in our study. Since genotype-specific changes were found in HBsAg levels during pegylated interferon treatment [36], it would be interesting to determine the effect of genotype on the kinetics of viral protein production after HBsAg seroclearance. Baseline HBV DNA levels were not available in our present study. A previous study in Asian CHB patients found low HBV DNA levels to be predictive of eventual HBsAg seroclearance [2], and therefore, such data should be included in future longitudinal studies concerning these two serologic markers.

In conclusion, the detection of HBcrAg and linearized HBsAg in more than 40% of CHB patients achieving HBsAg seroclearance documented by a conventional HBsAg assay suggests that transcription of viral proteins still exists even when serum HBsAg is undetectable. These two novel assays can assist the diagnosis of CHB patients with prior HBsAg seroclearance. Further longitudinal studies are needed to determine if these two serologic markers have prognostic implications for both treated and untreated CHB patients.

Conflict of interest No conflicts of interest exist for all authors.

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III. B 型肝炎

B型肝炎の病態

HBV遺伝子型と臨床像

Clinical implication of hepatitis B virus genotype

新海 登[1]　田中靖人[2]

Key Words：HBV遺伝子型(genotype)，遺伝子変異

はじめに

B型肝炎患者は無症候性キャリアも含めて現在，全世界で3億5千万人と推定され，世界中で年間約100万人がB型肝炎ウイルス(HBV)感染に関連して死亡している．我が国においてはHBV感染症例は150万人前後存在するとされている．1986年から母児感染予防事業によりB型肝炎ワクチン，抗B型肝炎ヒト免疫グロブリンの投与が行われ，ほぼ完全に垂直感染を抑えることに成功した．また，核酸増幅検査導入により輸血後B型肝炎は極めてまれになった．しかし近年性行為による水平感染が増加しており，その中で従来self-limitingな疾患であると考えられていた急性肝炎患者の慢性化が報告されるようになり，問題となっている．更に，B型肝炎患者の中には様々な治療を行ってもなかなか奏効せず，病状が悪化，進行する例も少なくない．このように臨床の場において同じHBV感染者であるにもかかわらず，その経過においては大きな差があることがしばしば経験されている．その原因の一つとしてHBV遺伝子型(genotype)が注目されている．

そこで本稿では，HBV genotypeによる臨床像の違いについて述べる．

1. HBV genotype

一般的にDNAウイルスはRNAウイルスに比べて遺伝子変異が少ないとされているが，HBVは逆転写過程をもつために高率に変異を起こすことが知られており，DNAウイルスの中では変異しやすいウイルスと考えられている．

HBVは近年の分子系統解析の進歩に伴い，現在までにgenotype A型からJ型までの10種に分類されている．これらのHBV genotype分布には地域特異性が存在し，genotype A，Dはヨーロッパ諸国および地中海沿岸に広く分布する一方，genotype B，Cは東アジアを中心に広く分布している．またgenotype Eは主に西アフリカに分布し，genotype F，Hは中南米に分布している．genotype Gは今のところフランス，ドイツ，USなどの一部の地域においてのみ報告されている．

日本全国13施設の共同研究により解析可能であった720例のHBV genotypeの分布状況は，genotype Cが約85％，genotype Bが約12％，genotype Aが約2％であり，我が国における持続感染者はgenotype Cとgenotype Bがほとんどである．日本国内においてもこの2つのgenotype間には地域特異性があり，ほとんどの地域ではHBV/Cが大多数を占めるのに対し，

[1]Noboru Shinkai: Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences　名古屋市立大学大学院医学研究科 消化器・代謝内科学　[2]Yasuhito Tanaka: Department of Virology and Liver Unit　同 病態医科学

0047-1852/11/¥60/頁/JCOPY

沖縄と東北地方ではHBV/Bの割合が非常に高い[1]．近年，我が国においてB型急性肝炎が増加傾向であり，なかでもgenotype Aの増加が目立っている．成人のHBV初感染による急性肝炎は，我が国ではself-limitingな疾患であり慢性化はまれであると考えられていたが，欧米では急性肝炎の約10％が慢性化しており，これがHBV genotypeの違いによることがわかってきている[2]．欧米にみられていたgenotype Aの初感染からの慢性化が我が国においても報告されるようになり，現在genotype Aによる慢性肝炎の増加が報告されている[3]．

一方でgenotype間の病態の違いに関してはそのgenotypeの地域分布に偏りがあるため，すべてのgenotypeを横断的に検討することは困難である．

またgenotypeと病態に関しては，genotypeに依存した頻度の多い変異が存在している．病態の一部には変異が関与していると思われるため，それを踏まえて記述する．

2. Genotype Bおよびgenotype C

前述のとおり，我が国におけるHBVは主にgenotype BとCである．我が国の持続感染のほとんどは母児感染による垂直感染がほとんどであり，思春期頃よりALTの上昇をきたす．そして，HBe抗原陽性からHBe抗体陽性へとセロコンバージョンを起こし，ウイルスの排除には至らないものの，持続感染のまま病態は安定化する．しかし一方で，セロコンバージョンを起こさず肝炎が持続し，肝硬変，肝癌，あるいは肝不全へと病状が進行してしまう例や，セロコンバージョンを起こしたにもかかわらず肝炎が持続する例もみられる．HBV genotype別に検討してみると，genotype Bの患者は比較的若年からセロコンバージョンを起こし，無症候性キャリアとなりやすいが，genotype Cの患者はHBe抗原陽性のまま病態が進行してしまう例が多いことがわかる[4]．

我が国においてはgenotype Bの予後は良好であるが，一方台湾では若年肝癌がHBV/Bで多く[5]，同じgenotypeであるにもかかわらず病態に大きな相違がみられた．そこで我が国を含むアジアから得られた検体を用いてgenotype Bの全塩基配列を決定し比較してみると，アジア諸国に分布するsubgenotype Ba(Asia型)と我が国固有のsubgenotype Bj(Japan型)の2つの亜型が存在することが確認できた．面白いことに，subgenotype Baはプレコアからコア領域の部分がgenotype Cとウイルス遺伝子組換えを起こしていた[6]．

これらsubgenotype Ba，subgenotype Bj，genotype C(C2/Ce(極東アジア型))について，年齢，性別，病態をマッチさせたケースコントロールスタディを行ったところHBe抗原陽性率はHBV/C(C2/Ce)で最も高く，HBV/Bjが最も低かった．更に，HBe抗原産生に関連しているウイルス遺伝子変異の検討を行った結果，basal core promoter(BCP)の2重変異(A1762T/G1764A)はHBV/Cで高率に認められ，HBV/Bjは最も低率であった．逆にプレコア変異(G1896A)はgenotype Bjで高率であり，genotype Cは低率であった．subgenotype Baはsubgenotype Bjとgenotype Cのちょうど中間的な性質を示していた[7](**表1**)．subgenotype Baはgenotype Cとのウイルス遺伝子組換えによりgenotype Bとgenotype Cの両方の性質を獲得したのかもしれない．

我が国における劇症肝炎と急性肝炎のケースコントロールスタディでは，劇症肝炎に関与する因子にgenotype Bjであることや，genotype Bjに頻度が高いG1896A変異が報告されている[8]．またgenotype Baに関してもA1762T/G1764A，G1896A変異が劇症肝炎に関係する可能性[9]がある．なお，genotype Cとのウイルス遺伝子組換えを起こしていないgenotype BはBj以外に北極圏にみられるsubgenotype B6がある．その臨床的特徴はsubgenotype Bjとよく似ていた[10]．

また慢性肝炎の急性増悪に関しては，subgenotype BaにおいてはA1762T/G1764Aが，genotype C2/CeにおいてはT1753V，A1762T/G1764A，G1896A，G1899Aが有意差をもって慢性肝炎に対して頻度が高かった[11]．培養細胞

表1 HBV genotype BとCの特徴

	HBV genotype		
	Bj	Ba	C
HBe抗原陽性率	18%	35%	50%
コアプロモーター変異(nt.1762/1764)	15%	33%	63%
プレコア変異(nt.1896)	50%	18%	13%
ATG initiator(nt.1809-1812)	CCAC	CCAC	CCAC
encapsidation signal(nt.1858)	T	T	T

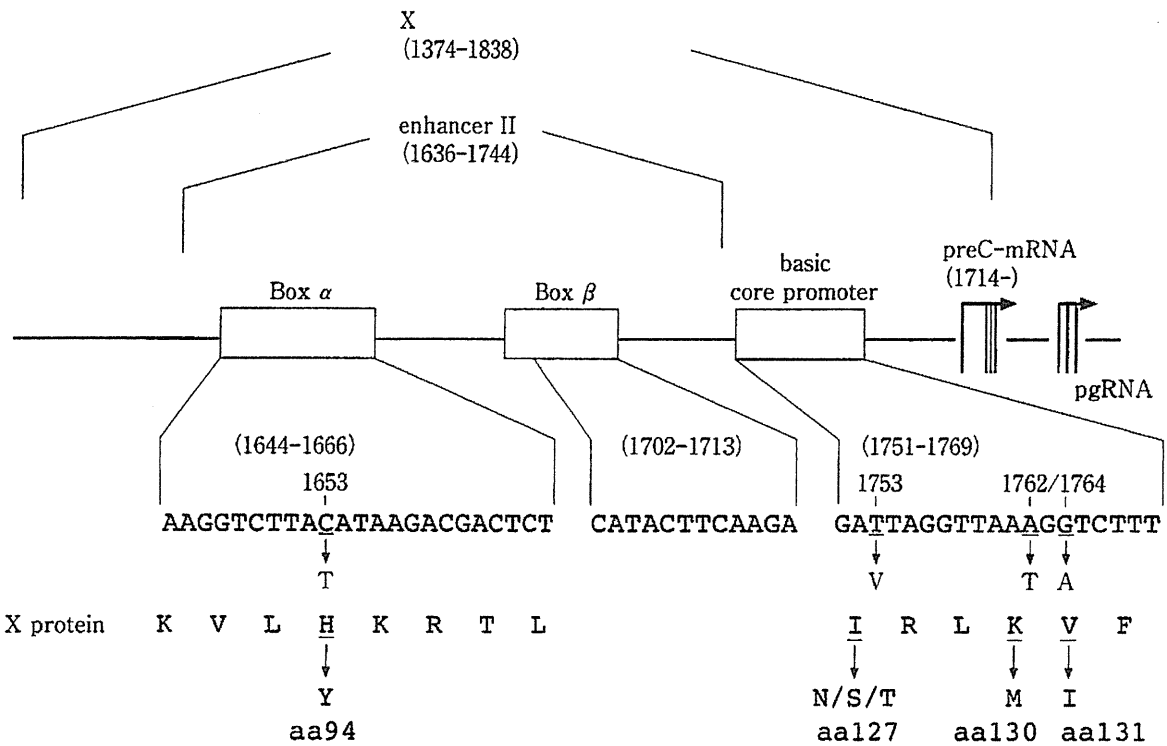

図1 HBV subgenotype C1/Cs(東南アジア型)とC2/Ce(極東アジア型)における enhancer II/コアプロモーター，プレコア領域の遺伝子変異

株とヒト肝細胞置換uPA/SCIDマウスを用いたHBV genotype A，B，Cの感染実験において，genotype Cの感染マウスに肝線維化の進展がみられたように，genotype CはBに比べて肝硬変，肝癌への進展がみられることが多い[12]．実際に台湾のコホートでもgenotype Cはgenotype Bの2倍の発癌リスクが示された[13]．我が国のHBV関連肝癌のgenotype分布をみると，多くがgenotype Cであり，subgenotype Bjは高齢者肝癌の一部に散見されるのみであった[14]．

genotype BとCを主とした肝発癌に関係するHBVの遺伝子変異はpre-S欠失，C1653T，T1753V，A1762T/G1764A変異がメタアナリシスで報告されている．

これらの変異の頻度をgenotype BとCとで比較してみると，Cにおいて頻度が高くなっている．subgenotype C2の遺伝子変異と肝癌との関係については様々な報告があるが，代表的なものとしてはBCP変異(でありHBXタンパクのアミノ酸変異でもある(図1))のC1653T，A1762T/G1764A変異がある[15-17]．また，subgenotype C2を主とした集団において経時的に

表2 HBV genotype AとCの特徴

	HBV genotype		
	Aa	Ae	D
HBe抗原陽性率	31%	49%	37%
コアプロモーター変異(nt.1762/1764)	50%	44%	25%
プレコア変異(nt.1896)	0%	0%	48%
ATG initiator(nt.1809-1812)	TCCT	CCAC	CCAC
encapsidation signal(nt.1858)	C	C	T

検討すると，A1762T/G1764A変異は必須であるがT1653やV1753が加わることで発癌にかかわるとする報告もある[18]．

HBVのpre-S欠失に関しては，HBVのpre S-S大タンパク質を過剰発現するトランスジェニックマウスで肝障害を起こし，肝細胞癌が多発することが報告されている[19]．pre-S欠失はgenotype Bよりgenotype Cに多くみられ，しかも病態進展(肝硬変，肝細胞癌)に有意に関与していることが報告されている[20]．すなわち，pre-S欠失はgenotypeにより差がみられ，病態の進行に伴い頻度が増加しており，genotypeによる病態の違いの原因の一つである可能性が考えられた．

3. genotype Aおよびgenotype D

我が国において今後HBV/Aの水平感染による慢性肝炎の増加が危惧されることは前に述べた．我が国に主に存在していたHBV genotypeはBjとCであり，これらの水平感染の場合では慢性化をきたすことは少ない．Yotsuyanagiらの報告によると，HBV/AによるB型急性肝炎の肝障害の程度はHBV/Cと比較して軽いが，HBs抗原の消失までの期間も長く，HBV/Aの慢性化率はHBV/Cと比較して高くなる傾向にあることを報告した[21]．またSuzukiらは，genotype Aの急性肝炎後の慢性化は31例中7例(23%)と高頻度に認めたと報告している[22]．我が国における多施設共同研究においても同様に遺伝子型Aの高い慢性化率を報告している[2,22,23]．このように，我が国におけるgenotype Aの急性肝炎の増加と他の遺伝子型と比較して慢性化率が高いことから，今後はgenotype Aのキャリアの増加が予想される[3]．

HBV/Aは欧州，米国，アフリカ，インド，フィリピンなど広く分布しており，世界で最も感染者が多いといわれている．一方，HBV/Dも地中海を主として世界に広く分布している．genotype Aは更にアフリカや東南アジアに分布するsubgenotype Aa(Asia/Africa型)と，欧米に広く存在するsubgenotype Ae(Europe型)の2つの亜型に分類される[24]．このsubgenotype Aaとsubgenotype Aeの間にもsubgenotype Ba，subgenotype Bjと同様に臨床的な違いが認められる．subgenotype Aa感染者は比較的若年でセロコンバージョンする一方で[25]，アフラトキシンの修飾の可能性もあるが肝癌の若年発症が高率に認められる[26]．subgenotype Aeは，成人の初感染による急性肝炎後の慢性化が約10%に認められるが，肝癌の発生は少ないとされる．

subgenotype Aaとsubgenotype Aeのウイルス学的特徴を明らかにするために，欧米に多いgenotype Dを加えたcase studyを行い，背景および臨床データを比較検討した．その結果，subgenotype Aaはsubgenotype AeやHBV/Dに比べHBe抗原陽性率が有意に低く，特に30歳未満でその傾向が顕著に認められた[27](表2)．subgenotype Aaではプレコア/コア領域の直前にあり，プレコア/コアタンパクの翻訳を制御しているATGイニシエーター(Kozak配列)に特異的な変異がみられ，そのためHBe抗原前駆体タンパクの翻訳効率が非常に低下したと考えられる[27,28]．

また，genotype Aはgenotype Dに比べ，プレ

コア領域変異を起こしにくいといわれているが，この理由としてεループと呼ばれる部位の2次構造にみられる特徴のためと考えられる．この立体構造はDNA合成のイニシエーションとなる部位で，途中で折り返すと多くの塩基が互いに相補的となり，ペアリングを形成して安定な形態をとる．この部分に変異が起こると立体構造が不安定化しDNAの複製効率が非常に低下することがわかっている．このループの中のプレコア領域1,896番の塩基は1,858番と対になっており，subgenotype Aa，Aeでは1,858番がCのため1,896番はGの方が安定でありAには変化しにくくなっている[28,29]．したがってsubgenotype Aa，Aeではプレコア領域変異を起こしにくいことがわかる．

またgenotype Aだからといって肝癌にならないわけではなく，我が国のgenotype Aの慢性肝炎においての検討ではHBe抗原のセロコンバージョン率が低く，肝硬変や肝癌の発症率もgenotype Bよりも高い可能性がある[3]．genotype AとDの比較では，慢性肝炎においてHBe抗原のセロコンバージョン後の生化学的な消退やHBV DNAの陰性化，HBs抗原の陰性化はgenotype Aで頻度が高い[30]．

genotype Dは比較的若年でプレコア変異によりHBe抗原のセロコンバージョンを起こす．遺伝子変異と病態に関しては，G1764T/C1766GはBCP変異の中で頻度が高く(29.2％)，HBe抗原陰性例の39.3％にみられた．A1752CやT1753V変異は肝癌患者に有意差をもって多く認められ，更にgenotype DにおいてA1762T/G1764A変異はもともと頻度が低いが，血中HBVウイルス量が高値な肝癌患者にはみられた[31]．また，genotype Dに関してはsubgenotype D1-D5において変異のパターンが異なるとの報告もあり[32]，今後の検討が待たれる．

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原　　著

HBs抗原定量値の臨床的有用性の検討

中　村　恵利那*1　角　田　浩　一*2　松　浦　健太郎*3
菅　内　文　中*4　新　海　　登*5　田　中　靖　人*6

Evaluation of the Clinical Utility of Quantitative Measure of Hepatitis B Surface Antigen

*Erina NAKAMURA**1, *Hirokazu KAKUDA**2, *Kentaro MATSUURA**3,
*Fuminaka SUGAUCHI**4, *Noboru SHINKAI**5 and *Yasuhito TANAKA**6,

There are 1.5 million hepatitis B virus (HBV)-infected patients in Japan. Anti-viral therapy is important for chronic hepatitis B patients to prevent hepatocellular carcinoma. Recently, HBs antigen (HBsAg) quantification has been reported to be useful for not only HBV screening but also for monitoring of anti-viral treatment. In this paper, we evaluated the clinical utility of quantitative assay of HBsAg by HISCL HBsAg kit. Although there can be a significant difference in age, HBeAg positive/negative and viral genotype, there is not in the disease stage. Moreover, the weak correlation was confirmed between HBsAg and HBV-DNA levels with or without anti-virus treatment. In the clinical practice, as HBV-DNA becomes undetectable immediately by anti-viral therapy such as entecavir, it may be difficult to evaluate the efficacy. The monitoring of the HBsAg concentration in addition to HBV DNA would be useful for the evaluation. Hence, the clinical role of HBsAg concentration could spread widely in Japan.

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Corresponding author: *Erina NAKAMURA*, Scientific Research Division, Scientific Affairs, Sysmex Corporation, Kobe 651-2241, Japan. E-mail: Nakamura.Erina@sysmex.co.jp

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B型肝炎ウイルス(HBV)感染者は全世界で4億2千万人，日本においては150万人程度存在するといわれている。アジアではHBVは主に母子感染によって感染し，慢性化，その後肝癌へ進展する症例も多く，肝炎が持続する患者においては，積極的な治療介入が必要である。現在，B型慢性肝炎，肝硬変の治療法としては，インターフェロン，核酸アナログ製剤による抗ウイルス療法が中心となっている。

近年，HBs抗原量とB型肝炎の病態や治療効果の関連性が報告されており，スクリーニング目的だけでなく，HBVキャリアの治療モニター・効果予測のマーカーとしても注目されている。既にアーキテクト・HBsAgQT(アボットジャパン㈱)が臨床応用されている[1]。2007年に化学発光免疫測定法(CLEIA

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*1,2 シスメックス株式会社学術部(〒651-2241 神戸市西区室谷1-3-2)
*3~6 名古屋市立大学大学院医学系研究科病態医科学，中央臨床検査部，
*3~5 同　大学院医学系研究科消化器・代謝内科学分野(〒467-8601 名古屋市瑞穂区瑞穂町字川澄1)

法)を測定原理とした，高感度かつ広い測定範囲で定量可能なHISCL HBsAg試薬を開発した。既に基礎的検討がいくつか行われており[2]，日常検査に有用であることが確認されている。我々は，今回HBs抗原定量値の臨床的有用性について知見を得たので報告する。

I. 対象および方法

A. 対　象

名古屋市立大学病院およびその関連施設に通院中のB型慢性肝疾患患者313例(無症候性キャリア[ASC] 81例，慢性肝炎[CH] 132例，肝硬変[LC] 30例，肝細胞癌[HCC] 33例，不明37例)を対象とした。患者背景については**Table 1**に示す。治療の有無については，過去あるいは検体採取時にインターフェロン治療，核酸アナログ製剤による治療歴を有するものを「抗ウイルス療法あり」とした。なお，本研究は，担当医より対象者に文書によって説明，同意を取得した後，実施した。

B. 方　法

HBs抗原定量値は，全自動免疫測定装置HISCL-2000i専用試薬であるHISCL HBsAg試薬(シスメックス㈱)にて測定した。本試薬は最小検出感度0.03 IU/mLと高く，測定範囲が0.03～2,500 IU/mLと広いことが特徴である。今回，0.03 IU/mL以上を陽性と判定し，測定範囲を超えた検体については，自動希釈機能により40倍希釈を行い，測定した。

また，HBV Genotypeはイムニス HBV ゲノタイプEIA(㈱特殊免疫研究所)，HBV-DNAはアンプリコア法(ロシュ・ダイアグノスティックス㈱)またはTMA法にて測定を行った。

II. 結　果

A. 年齢別のHBs抗原量

B型慢性肝疾患患者313例のうち，年齢，治療の有無についての情報を有する280例について，治療群および非治療群において年齢別にHBs抗原量を比較した。治療群，非治療群ともに40歳未満と50代，40歳未満と60歳以上，40代と60歳以上で有意差が見られ，高齢になるに従いHBs抗原量は低値となる傾向がみられた(**Fig. 1**)。

B. HBe抗原別のHBs抗原量

治療の有無についての情報を有するHBe抗原陽性例70例とHBe抗原陰性例205例について，HBs抗原量を比較したところ，治療の有無に拘らず，HBe抗原陰性群の方が有意にHBs抗原量が低い結果となった(**Fig. 2**)。

C. 病態別のHBs抗原量

病態(ASC，CH，LC，HCC)が把握できている276例においてHBs抗原量を比較したところ，有意差はみられなかった(**Fig. 3**)。

D. HBV-DNA量とHBs抗原量

治療の有無別に，HBV-DNA量(Log copies/mL)とHBs抗原量(Log IU/mL)の情報がある260例(治療群111例，未治療群149例)について，両者の相関関係を検討した。治療群は$y=0.130x+2.71$，$r=0.267$，未治療群は$y=0.302x+1.48$，$r=0.457$となり，未治療群にのみ弱い正の相関性がみられた(**Fig. 4**)。治療群においては抗ウイルス療法によりHBV-DNAは減少するがHBs抗原量の減少までは得られない症例が多いために相関がみられなかった。

E. Genotype別のHBs抗原量

Genotype(以下，GT)が判定可能であった症例は290例であり，内訳はGT-A 10例，GT-B 68例，

Table 1　Characteristics of chronic hepatitis B patients

Male : Female	181 : 132
Age	54±14
Genotype (A, B, C, D, unknown)	10, 68, 206, 6, 23
Disease Stage (ASC, CH, LC, HCC, unknown)	81, 132, 30, 33, 37
ALT	50±109
HBV-DNA (median, log copies/mL)	4.4
HBeAg　(+) : (−) : unknown	72 : 207 : 34
HBeAb　(−) : (+) : unknown	64 : 214 : 35
Anti-viral treatment : no treatment : unknown	118 : 177 : 18

※ They are only the known data.

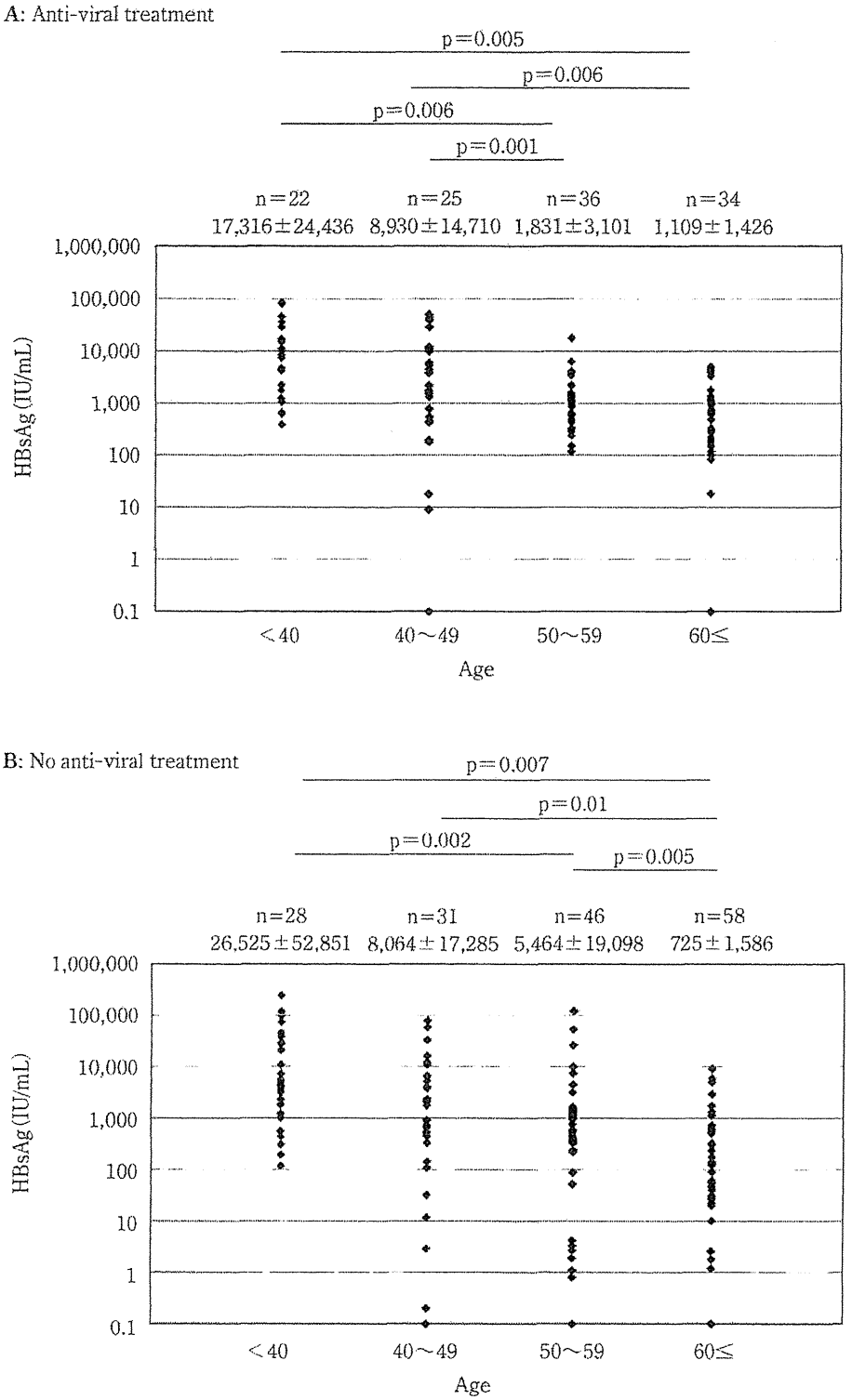

Figure 1　Quantity of HBsAg according to the age.

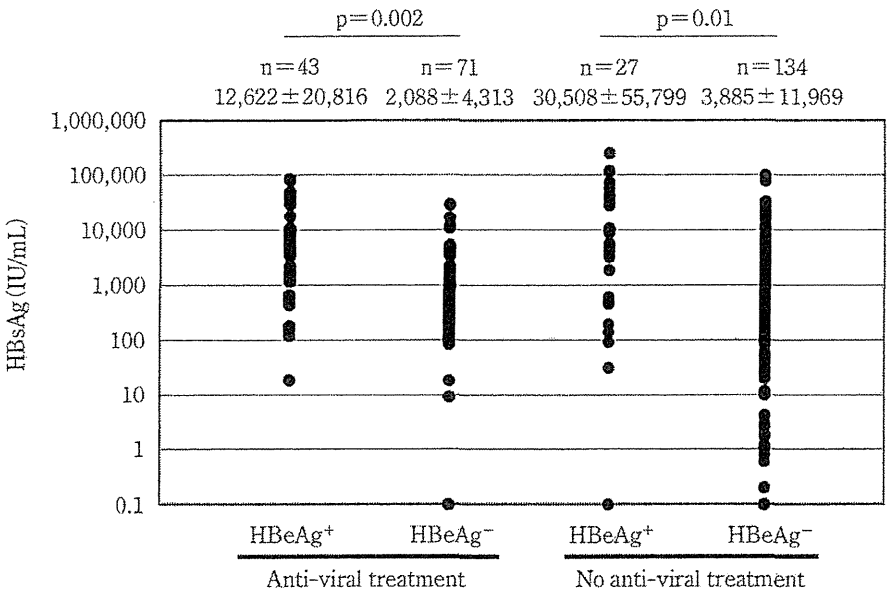

Figure 2 Quantity of HBsAg according to HBeAg status.

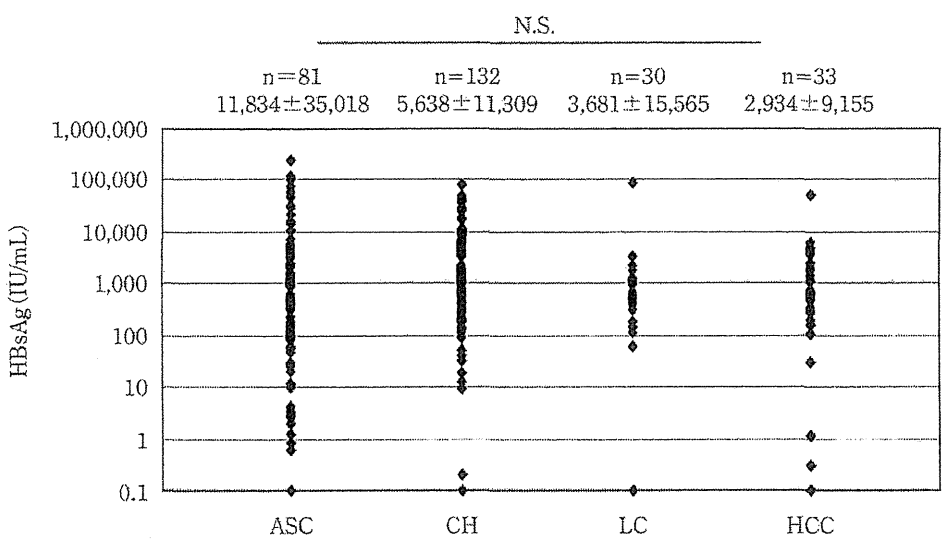

Figure 3 Quantity of HBsAg according to Disease Stage.

GT-C 206例，GT-D 6例であった。これら4群でHBs抗原量を比較したところ，GT-A＞GT-D＞GT-C＞GT-Bの順でHBs抗原量が高い結果となり，GT-AとC，CとBの間において有意差をみとめた(**Fig. 5**)。

III. 考　　察

現在，HBs抗原測定は定性検査として用いられることが多く，感染の有無を確認するスクリーニングが主な測定目的である。しかし，最近ではHBs抗原を定量検査として測定できる試薬がいくつか発売されており，B型肝炎の病態や治療効果との関連性も報告されている。これまでに，HBs抗原の定量測定が可能な試薬に関し，基礎的検討がいくつか報告され，日常検査において有用であることが確認されている[2]。そこで，今回，HBs抗原定量値の臨床的有用性について検討した。

年齢別にHBs抗原量を比較したところ，治療の有無に拘らず高齢になるにつれてHBs抗原量は低値となる傾向がみられ，年齢との関連性が明らかとなった。Genotype別の比較検討では，GT-A＞D＞C＞Bの順で高値である傾向がみられた。近年，本邦において従来外来株であったGT-AによるB型急性肝炎が特に若年者において増加しており，慢性化率

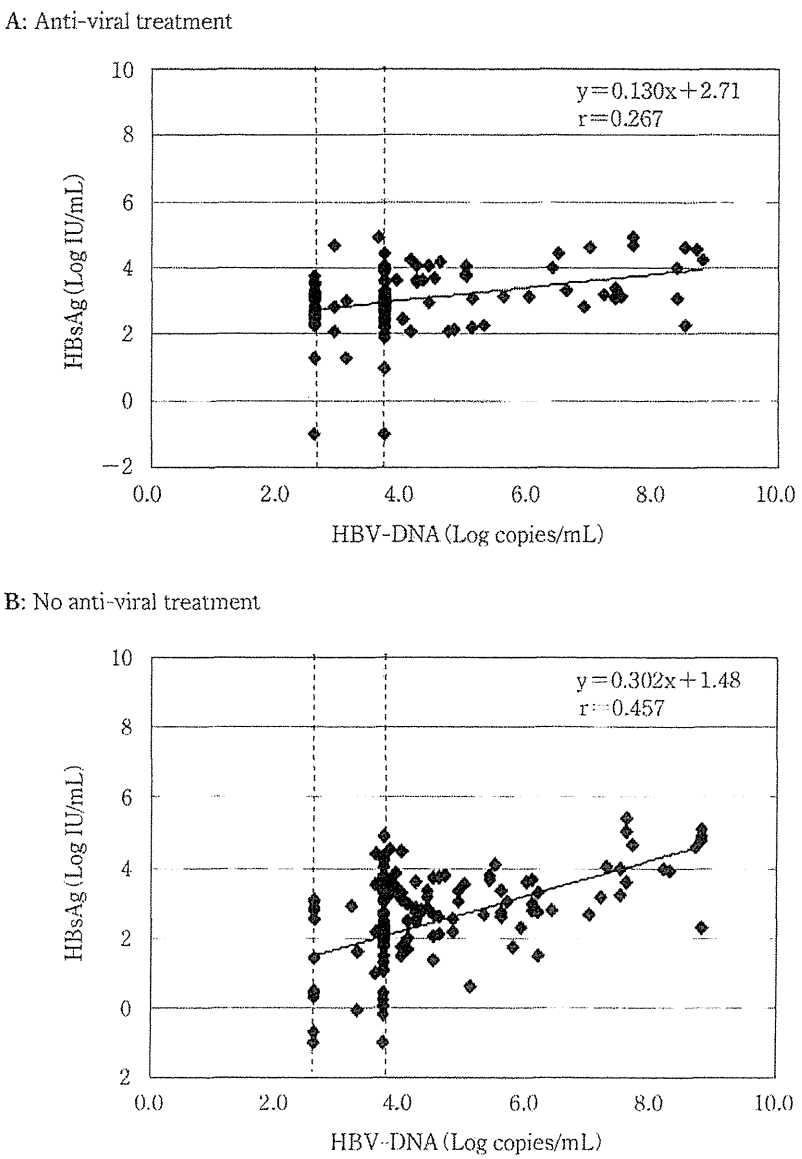

Figure 4　The correlation between HBsAg and HBV DNA.

が高いことが特徴で，実際にB型慢性肝疾患患者においてもGT-Aの割合が増加していることが報告されている[1]。したがって，GT-Aの患者は比較的若年である例が多く，HBs抗原量が高値であることについては年齢が影響している可能性が考えられる。今後，多数のGT-A例において，他のgenotypeと年齢をマッチさせHBs抗原量を比較検討する必要性がある。

HBe抗原別の検討では，治療の有無に拘らずHBe抗原陽性群が陰性群に比べてHBs抗原量が有意に高い結果となった。一般的にHBe抗原陽性例はHBVの増殖力が強いため血中のウイルス量が多いことが多く，HBs抗原量も高いということは妥当な結果であると考える。

HBV-DNA量とHBs抗原量の相関関係を検討したところ，未治療群にのみ弱い正の相関性が認められた。治療群ではHBV DNAが検出感度以下にも拘らずHBs抗原量が高い症例を多くみとめた。核酸アナログ製剤やインターフェロン投与により，HBV-DNAは速やかに減少する症例が多いが，HBs抗原量が短期間で減少する例は多くはない。そのため，HBV-DNAのモニタリングだけでは，治療効果判定が難しく，HBs抗原定量値を経時的に把握する重要性も報告されている[3]。また，インターフェロン治療中のHBs抗原定量値をモニタリングすることで治療効果の予測が可能であるとの報告もあり[4]，

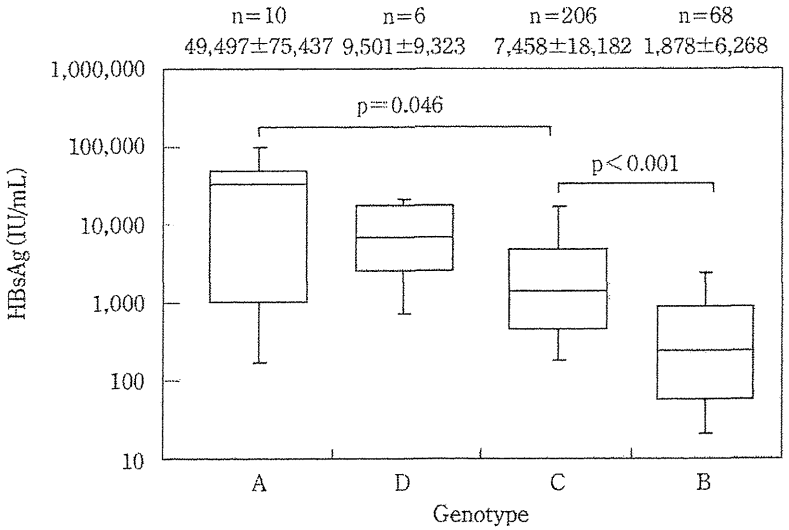

Figure 5 Quantity of HBsAg according to genotype.

国際的にはHBs抗原定量値が広く用いられている。今後，本邦でも広くHBs抗原定量値が測定されるようになることが予想される。

IV. ま と め

今回，HISCL HBsAg試薬を用い，HBs抗原定量値の臨床的有用性について検討した。病態別の検討では差が認められなかったものの，年齢，HBe抗原，Genotype別の検討ではHBs抗原定量値に有意差を認めた。今後，さらに多数例でHBs抗原定量値の臨床的意義，有用性について検討がなされ，抗ウイルス療法の効果判定，予後予測などに利用されていくものと考えられる。

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection

Mika Kurokawa · Naoki Hiramatsu · Tsugiko Oze · Takayuki Yakushijin · Masanori Miyazaki · Atsushi Hosui · Takuya Miyagi · Yuichi Yoshida · Hisashi Ishida · Tomohide Tatsumi · Shinichi Kiso · Tatsuya Kanto · Akinori Kasahara · Sadaharu Iio · Yoshinori Doi · Akira Yamada · Masahide Oshita · Akira Kaneko · Kiyoshi Mochizuki · Hideki Hagiwara · Eiji Mita · Toshifumi Ito · Yoshiaki Inui · Kazuhiro Katayama · Harumasa Yoshihara · Yasuharu Imai · Eijirou Hayashi · Norio Hayashi · Tetsuo Takehara

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Abstract
Background Nucleotide analogues have recently been approved for the treatment of patients with hepatitis B virus (HBV) infection. However, it is still controversial whether the decrease of HBV-DNA amount induced by treatment with nucleotide analogues can reduce the risk of hepatocellular carcinoma (HCC) development in HBV patients.

Methods A total of 293 HBV patients without HCC who were treated with lamivudine (LAM) were enrolled in a multicenter trial. The incidence of HCC was examined after the start of LAM therapy, and the risk factors for liver carcinogenesis were analyzed. The mean follow-up period was 67.6 ± 27.4 months.

Results On multivariate analysis for HCC development in all patients, age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, cirrhosis, and median HBV-DNA levels of ≥4.0 log copies/ml during LAM treatment were significant risk factors. The cumulative carcinogenesis rate at 5 years was

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M. Kurokawa · N. Hiramatsu (✉) · T. Oze · T. Yakushijin · M. Miyazaki · A. Hosui · T. Miyagi · Y. Yoshida · H. Ishida · T. Tatsumi · S. Kiso · T. Kanto · A. Kasahara · T. Takehara
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: hiramatsu@gh.med.osaka-u.ac.jp

S. Iio
Higashiosaka City General Hospital, Higashiosaka, Japan

Y. Doi
Otemae Hospital, Osaka, Japan

A. Yamada
Sumitomo Hospital, Osaka, Japan

M. Oshita
Osaka Police Hospital, Osaka, Japan

A. Kaneko
NTT West Osaka Hospital, Osaka, Japan

K. Mochizuki · H. Hagiwara · N. Hayashi
Kansai Rosai Hospital, Amagasaki, Japan

E. Mita
National Hospital Organization Osaka National Hospital, Osaka, Japan

T. Ito
Osaka Kouseinenkin Hospital, Osaka, Japan

Y. Inui
Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan

K. Katayama
Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan

H. Yoshihara
Osaka Rousai Hospital, Sakai, Japan

Y. Imai
Ikeda Municipal Hospital, Ikeda, Japan

E. Hayashi
Kinki Central Hospital of Mutual Aid Association of Public School Teachers, Itami, Japan

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3% in patients with chronic hepatitis and 30% in those with cirrhosis. For the chronic hepatitis patients, the log-rank test showed the significant risk factors related to HCC development to be age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, and hepatitis B e antigen negativity, but median HBV-DNA levels of <4.0 log copies/ml (maintained viral response, MVR) did not significantly suppress the development of HCC. In cirrhosis patients, however, the attainment of MVR during LAM treatment was revealed to reduce the risk of HCC development.

Conclusions These results suggest that the incidence of HCC in HBV patients with cirrhosis can be reduced in those with an MVR induced by consecutive LAM treatment.

Keywords Lamivudine · Chronic hepatitis B · Cirrhosis · Hepatocellular carcinoma · HBV-DNA level

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
ADV	Adefovir
ETV	Entecavir
Hbs Ag	Hepatitis B surface antigen
PCR	Polymerase chain reaction
TMA	Transcription-mediated amplification
IVR	Initial viral response
MVR	Maintained viral response
HBe Ag	Hepatitis B e antigen
CT	Computed tomography
MRI	Magnetic resonance imaging
ALT	Alanine aminotransferase

Introduction

More than 350 million people worldwide suffer from chronic infection with hepatitis B virus (HBV) [1–3]. Chronic HBV infection eventually leads to the development of cirrhosis and hepatocellular carcinoma (HCC), and raises the risk of hepatic disease-related death [4–6]. In Japan, up to 15% of HCC patients are diagnosed with HBV-related liver disease [7].

HCC is one of the most common malignancies in Japan and its incidence has been increasing over the past 30 years. Recently, various treatments such as transcatheter arterial embolization/chemoembolization, radio-frequency ablation, and hepatic resection have been reported to yield significant improvements in overall patient survival [8–11]. However, HCC relapse has thus far been observed in a majority of treated patients due to its highly malignant potential. In this regard, successful treatment of chronic HBV infection should prevent the patient's liver from progressing to cirrhosis and reduce the risk of HCC development. In recent years, the treatment of chronic hepatitis has changed greatly with the development of various antiviral therapies with nucleoside/nucleotide analogues such lamivudine (LAM), adefovir (ADV), and entecavir (ETV) [12–15]. LAM has long been used against chronic hepatitis, and many reports have demonstrated that LAM is effective in stabilizing inflammatory activity, suppressing HBV-DNA replication, and improving liver histological findings in chronic hepatitis patients [16, 17] and in HBV-related cirrhosis patients [18]. Furthermore, LAM has been reported to reduce the incidence of HCC in patients with chronic hepatitis B [19]. However, it is still controversial whether or not treatment using nucleotide analogues can reduce the risk of HCC development in HBV-infected patients [20, 21], and the relationship between the effect of HBV suppression and HCC development during LAM treatment has not yet been discussed in detail. Also, the risk factors for HCC development in HBV-infected patients who have been treated with LAM have not been sufficiently evaluated. In this study, we aimed to clarify whether the decrease of HBV-DNA amount induced by LAM therapy could reduce the incidence of HCC in HBV-infected patients.

Patients and methods

Patient selection and study design

This study was conducted at Osaka University Hospital and other institutions participating in the Osaka Liver Forum in Japan. The subjects were 293 consecutive patients with HBV infection who underwent continuous LAM therapy for more than 24 weeks from September 2000 to September 2006. All patients tested positive for hepatitis B surface antigen (HBs Ag) or had detectable levels of HBV DNA in their sera according to findings from a polymerase chain reaction (PCR)-based method or a transcription-mediated amplification (TMA) method. Exclusion criteria were patients with anti-hepatitis C antibody, anti-human immunodeficiency virus antibody, and other liver diseases (alcoholic liver disease, drug-induced liver disease, and autoimmune hepatitis). Also excluded were patients with a history of HCC and those who developed HCC within the first 24 weeks of the follow-up period after the initiation of LAM therapy (because of the possibility that microscopic HCC had been present before the initiation of treatment).

All patients were treated with 100 mg of LAM daily. Of the 293 patients, 129 underwent ADV (10 mg/day) therapy in addition to receiving ongoing LAM treatment. For 43 patients who started ETV administration in lieu of LAM, the observation period was terminated when they started

ETV. LAM resistance was confirmed by virological breakthrough and was defined as an increase in serum HBV-DNA by >1 $\log_{10}$ greater than the nadir [22]. If virological breakthrough developed and alanine aminotransferase (ALT) was elevated over the upper normal limit, the patients received add-on ADV at 10 mg/day.

In this study, all patients were examined for serum HBV-DNA level just before therapy initiation and every 6 months during treatment. The initial viral response (IVR) was defined as HBV-DNA <4.0 log copies/ml in the first 24 weeks of the follow-up period after the initiation of LAM therapy, and the maintained viral response (MVR) was defined as median HBV-DNA levels of less than 4.0 log copies/ml measured every 6 months during therapy.

This study protocol followed the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

HBV testing

HBs Ag, hepatitis B e antigen (HBe Ag) and anti-hepatitis B e antibody (anti-HBe) levels were examined by chemiluminescence immunoassay or enzyme immunoassay. HBV DNA was measured by a PCR-based method (Amplicor HBV monitor; Roche Diagnostics, Tokyo, Japan) or a TMA method (TMA-HPA; Fujirebio, Tokyo, Japan), which have lower detection limits of 2.6 and 3.7 log copies/ml, respectively. The LAM-resistant YMDD mutant virus was examined by a PCR-ELMA method. Serum samples were stored frozen at −80°C.

Diagnosis of HCC and cirrhosis

Ultrasonography was carried out before LAM therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by computed tomography (CT), magnetic resonance imaging (MRI), or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT or MRI. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the patient's consent or the patient was carefully followed until a diagnosis was possible with definite observation by CT, MRI, or hepatic angiography. Cirrhosis was diagnosed by liver biopsy or laparoscopy, and for patients without this information, by clinical data, imaging modalities, and portal hypertension.

Statistical analysis

Quantitative variables were expressed as means ± SD. Quantitative variables at the baseline were compared among two groups, the chronic hepatitis and cirrhosis groups, using the Mann–Whitney *U*-test. Categorical data, such as gender and status of HBe Ag, were compared using Fisher's exact test. The cumulative incidence of HCC was evaluated with a Kaplan–Meiercurve and the differences between groups were analyzed by the log-rank test. For multivariate analysis to investigate factors affecting the cumulative incidence of HCC, Cox's regression analysis was carried out. A value of $p < 0.05$ (two-tailed) was considered to be statistically significant. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of patients

The baseline clinical features of the enrolled patients before LAM administration are shown in Table 1. The mean age of the patients was 48.0 ± 10.7 years, 214 (73%) of the entire group were male, and 163 (56%) tested positive for HBe Ag. Of the 293 patients, 205 (70%) were diagnosed as having chronic hepatitis and 88 (30%) as having cirrhosis. The median HBV-DNA level was 7.0 (range 3.0 to 8.5<) log copies/ml. At baseline, the aspartate aminotransferase (AST) level was 131 ± 151 IU/l, the ALT level was 203 ± 252 IU/l, the total bilirubin level was 1.2 ± 1.6 mg/dl, the albumin (Alb) level was 3.8 ± 0.5 g/dl, and the platelet count was 13.7 ± 5.4 × 10^4/mm^3. The mean follow-up period for all patients was 67.6 ± 27.4 months, with a range of 12–110 months from the start of LAM treatment. There were significant differences between patients with chronic hepatitis and those with liver cirrhosis in age, AST, ALT, total bilirubin, Alb, and platelet counts.

Cumulative incidence of development of HCC

Figure 1a shows the Kaplan–Meier curve of the cumulative HCC incidence for all HBV patients treated with LAM or LAM plus ADV. Of the 293 patients with HBV infection, 32 (10.9%) developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 15% at 7 years.

Figure 1b shows the Kaplan–Meier curve of the cumulative HCC incidence according to initial diagnosis (chronic hepatitis vs. cirrhosis). Eight (4%) of the 205 enrolled chronic hepatitis patients developed HCC and the cumulative carcinogenesis rate was 2% at 3 years, 3% at 5 years, and 5% at 7 years. On the other hand, 24 (27%) of the 88 enrolled cirrhosis patients developed HCC and the cumulative carcinogenesis rate was 15% at 3 years, 30% at 5 years, and 35% at 7 years.

Table 1 Patient characteristics

Factor	All	Chronic hepatitis	Cirrhosis	*p* value
Number of patients	293	205	88	
Age (years)	48.0 ± 10.7	46.3 ± 10.7	51.9 ± 9.8	<0.001**
Sex (male/female)	214/79	147/58	67/21	0.475
HBe Ag (positive	163 (56%)	121 (59%)	42 (48%)	0.068
HBV DNA (log copies/ml)[a]	7.0 (3.0 to 8.5<)	6.8±1.1	6.6 ± 1.1	0.162
AST (IU/l)	131 ± 151	143 ± 162	104 ± 120	0.045*
ALT (IU/l)	203 ± 252	235 ± 269	129 ± 189	<0.001**
Total bilirubin (mg/dl)	1.2 ± 1.6	0.9 ± 0.6	1.8 ± 2.7	<0.001**
Alb (g/dl)	3.8 ± 0.5	3.9 ± 0.4	3.5 ± 0.6	<0.001**
Platelets ($\times 10^4$/mm^3)	13.7 ± 5.4	15.6 ± 9.3	9.3 ± 3.8	<0.001**
Follow-up period (months)	67.6 ± 27.4	68.5 ± 26.5	65.5 ± 29.5	0.393

HBe Ag Hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin

[a] Values are expressed as medians

* $p < 0.05$, ** $p < 0.001$, comparing patients with chronic hepatitis and those with liver cirrhosis using the Mann–Whitney *U*-test for quantitative variables and Fisher's exact test for categorical variables

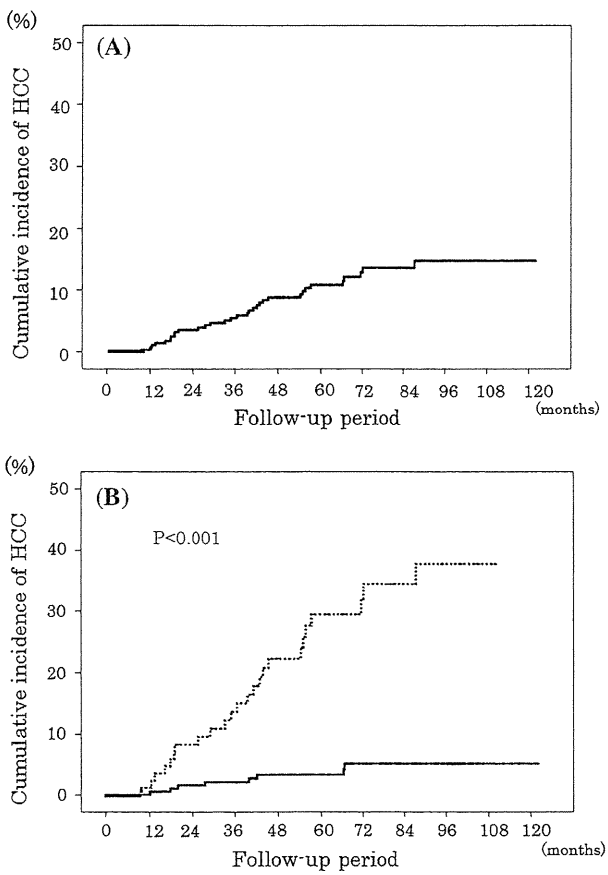

Fig. 1 Cumulative incidence of development of hepatocellular carcinoma (*HCC*) in patients with hepatitis B virus infection treated with lamivudine (LAM). **a** All cases; **b** chronic hepatitis or cirrhosis. *Solid line* Chronic hepatitis, *dotted line* cirrhosis

Risk factors for cumulative incidence of HCC development in all HBV-infected patients

Univariate analysis with the log-rank test was performed for all HBV-infected patients treated with LAM, with the results shown in Table 2. Univariate analysis with the log-rank test showed that the following were significant risk factors for the development of HCC: older age (≥50 years) ($p < 0.001$), cirrhosis ($p < 0.001$), high total bilirubin level (>1.2 g/dl) ($p = 0.004$), low Alb level (<3.8 g/dl) ($p = 0.019$), low platelet count (<14 × 10^4/mm^3) ($p < 0.001$), and non-MVR ($p = 0.035$).

Stepwise multivariate analyses of four of these variables were performed by Cox's regression analysis for all patients treated with LAM with the results shown in Table 3. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: age ≥50 years [hazard ratio (HR) 3.20, 95% confidence interval [CI] 1.08–9.53, $p = 0.036$], platelet count <14.0 × 10^4/mm^3 (HR 4.76, 95% CI 0.05–0.96, $p = 0.045$), cirrhosis (HR 4.64, 95% CI 1.75–12.4, $p = 0.002$), and non-MVR (HR 2.70, 95% CI 1.09–6.56, $p = 0.032$).

Cumulative incidence of and risk factors for HCC development in patients with chronic hepatitis and cirrhosis

The results of univariate analysis with the log-rank test for the development of HCC in chronic hepatitis patients treated with LAM are shown in Table 4, and the following were significant risk factors: older age (≥50 years) ($p = 0.002$), HBe Ag negativity ($p = 0.005$), and low platelet count (<14 × 10^4/mm^3) ($p = 0.004$). Suppression of median HBV-DNA levels to <4.0 log copies/ml by LAM treatment was not associated with the development of HCC in the chronic hepatitis patients. Only non-MVR (median HBV-DNA amount ≥4.0 log copies/ml) was shown to be a significant risk factor for the development of HCC in the cirrhosis patients ($p = 0.029$), while the factors of age, HBe Ag status, and platelet count were not significant in these patients (Table 4).