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CASE REPORT

A case of monocular blindness as the initial presentation of hepatocellular carcinoma with skull metastasis

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Abstract A 52-year-old man suffering from monocular blindness, with light perception only, was admitted to our hospital. The symptom had begun as low vision and developed rapidly within 3 weeks into monocular blindness in the right eye, with no other systemic manifestations. Imaging examinations revealed multiple hepatocellular carcinomas in the cirrhotic liver, and tumors at the skull base and vertebra. A pathological and immunochemical study of specimens obtained by endoscopic transnasal tumor biopsy and laminectomy revealed them to be metastatic hepatocellular carcinomas (HCCs). Although the patient underwent radiation therapy and chemotherapy, he died 5 months after admission to our hospital. The cranial HCC, involving only the optic canal, may have disturbed the optic nerve in preference to the other cranial nerves. This is the first report of a HCC patient with monocular blindness as the initial presentation of the disease.

Keywords Blindness · Hepatocellular carcinoma · Metastasis

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Introduction

Hepatocellular carcinoma (HCC), one of the most common types of cancer worldwide, is characterized by poor presentation of specific symptoms until advanced stages. The incidence of HCC metastases has been reported from <5 to 36.7% in clinical records [1–3] and more frequently in autopsy cases [4]. Bone is the third most frequent site for HCC metastasis followed by the lung and the abdominal lymph nodes. The common bone metastatic sites are the vertebra, the rib, and the long bone; however, bone metastasis of HCC to the skull is very rare. The frequent presentations of bone metastatic HCC are an occasional painful sensation, headache, weakness of limbs, seizures, and symptoms associated with a disturbance of the cranial nerves [3].

To the best of our knowledge, this is the first report of a HCC patient with monocular blindness as the initial presentation of the disease.

Case report

A 52-year-old man suffering from monocular blindness, with light perception only, was admitted to our hospital in April 2009. The symptom had begun as low vision and developed rapidly within 3 weeks into monocular blindness in the right eye, with no other systemic manifestations. The patient consumed excessive alcohol for over 30 years. He also had a medical history of blood transfusion for

Table 1 Laboratory data on admission

Hematology		*Serology test*	
White blood cells	5960/μl	HBs-antigen	Negative
Hemoglobin	14.9 mg/dl	HBs-antibody	Negative
Platelets	176 × 10^3/μl	HBc-antibody	Positive
		HBV-DNA PCR	2.1 log/ml
Blood chemistry		HCV-antibody	Negative
Total bilirubin	0.9 mg/dl		
Direct bilirubin	0.3 mg/dl	*Tumor markers*	
AST	68 U/l	AFP	1881 ng/ml
ALT	38 U/l	PIVKA-II	6911 mAU/ml
Alkaline phosphatase	581 U/l	CEA	2.22 ng/ml
γ-GTP	198 U/l	CA19-9	13.2 U/ml
Total protein	7.4 g/dl		
Albumin	3.9 g/dl	*Others*	
		ICG(15)	29%
Coagulation			
PT%	101%		

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* glutamyltransferase, *HBs* hepatitis B virus surface, *HBc* hepatitis B virus core antigen, *PCR* polymerase chain reaction, *AFP* α-fetoprotein, *PIVKA-II* protein induced by vitamin K absence, *CEA* carcinoembryonic, *CA19-9* carbohydrate antigen 19-9, *ICG(15)* indocyanine green 15 min retention rate

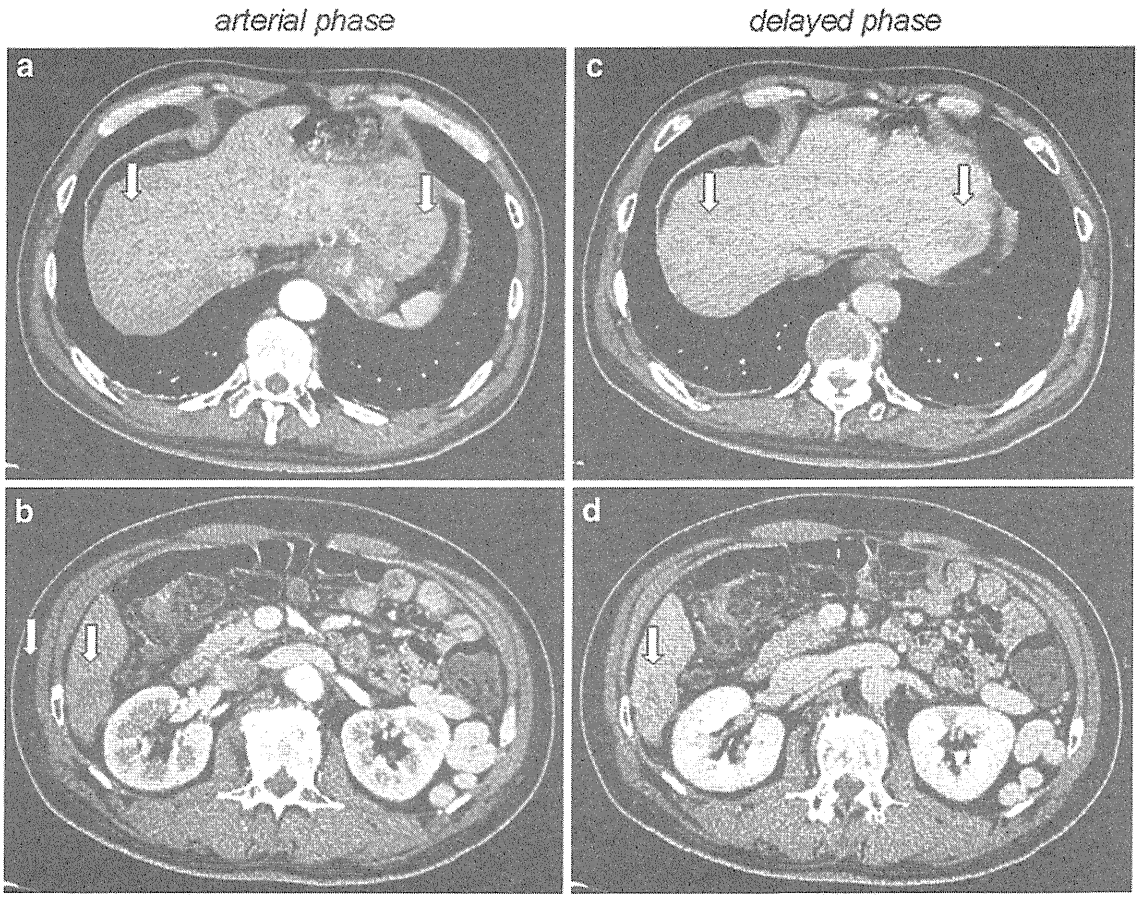

Fig. 1 Abdominal computed tomography scan on admission. *Arrows* indicate the mass in each scan

hemorrhagic gastric ulcer in 1990 and mitral valve plasty for mitral regurgitation in 2007.

Laboratory investigation on admission revealed negative for serum hepatitis B surface antigen; however, serum hepatitis B virus DNA (HBV-DNA) was substantively positive by polymerase chain reaction (PCR). Thus the liver dysfunction of this patient might be due not only to alcohol abuse but also to 'occult hepatitis B' infection.

Other laboratory findings were: total bilirubin 0.9 mg/dl (normal 0.2–1.3); serum aspartate aminotransferase 68 U/l (normal 13–33); alanine aminotransferase 38 U/l (normal 8–42); indocyanine green (ICG) 15 min retention rate 29.0% (normal 0–10). While serum levels of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9) were within normal range, tumor markers for HCC were elevated: α-fetoprotein (AFP) 1881 ng/ml (normal 0–6.2); protein induced by vitamin K absence (PIVKA-II) 6911 mAU/ml (normal 0–40) (Table 1). Enhanced computed tomography (CT) showed the 32 × 22 mm, 36 × 24 mm and 9 × 9 mm lesions, all of which were barely enhanced in the arterial phase study (Fig. 1a, b) and hypoenhanced in the portovenous study (Fig. 1c, d) in segments 2, 6 and 8 of the cirrhotic liver, respectively. Cranial magnetic resonance imaging (MRI) showed a tumor involving the optic canal spread across the right sphenoid sinus and sella turcica. It also showed the tumor to have slight high intensity on T1-weighted and low intensity on T2-weighted images. The tumor was enhanced homogeneously (Fig. 2a). Spinal MRI showed spinal tumors at cervical vertebrae (C1, C2), thoracic vertebrae (Th5, Th6) and sacrum (S1 and S2). The tumors of the thoracic vertebrae, in particular, seemed to press against the spinal cord intensively (Fig. 2b).

An endoscopic transnasal tumor biopsy was performed [5]. A microscopic examination of the specimen showed that the tumor was mainly composed of palisaded cells with yellow pigment-like bile (Fig. 3). After the patient was admitted to our hospital, leg paralysis emerged and developed rapidly. Therefore, emergency laminectomy with posterior fixation was performed [6]. Microscopic examination of the thoratic tumor specimen also showed palisaded tumor cells. The immunochemical expression of HepPar1 and cytokeratin (CK) 8 were positive [7–9] (Fig. 3). Taken together, both tumors were diagnosed as metastasis of moderately differentiated HCC.

The patient underwent radiation therapy: 3 Gy × 12 times for skull, 3 Gy × 12 times for cervical vertebrae, 3 Gy × 15 times for thoracic vertebrae; 3 Gy × 15 times for sacrum. He then underwent continuous infusion of 5-fluorouracil (750 mg on days 1–5), mitoxantrone (10 mg

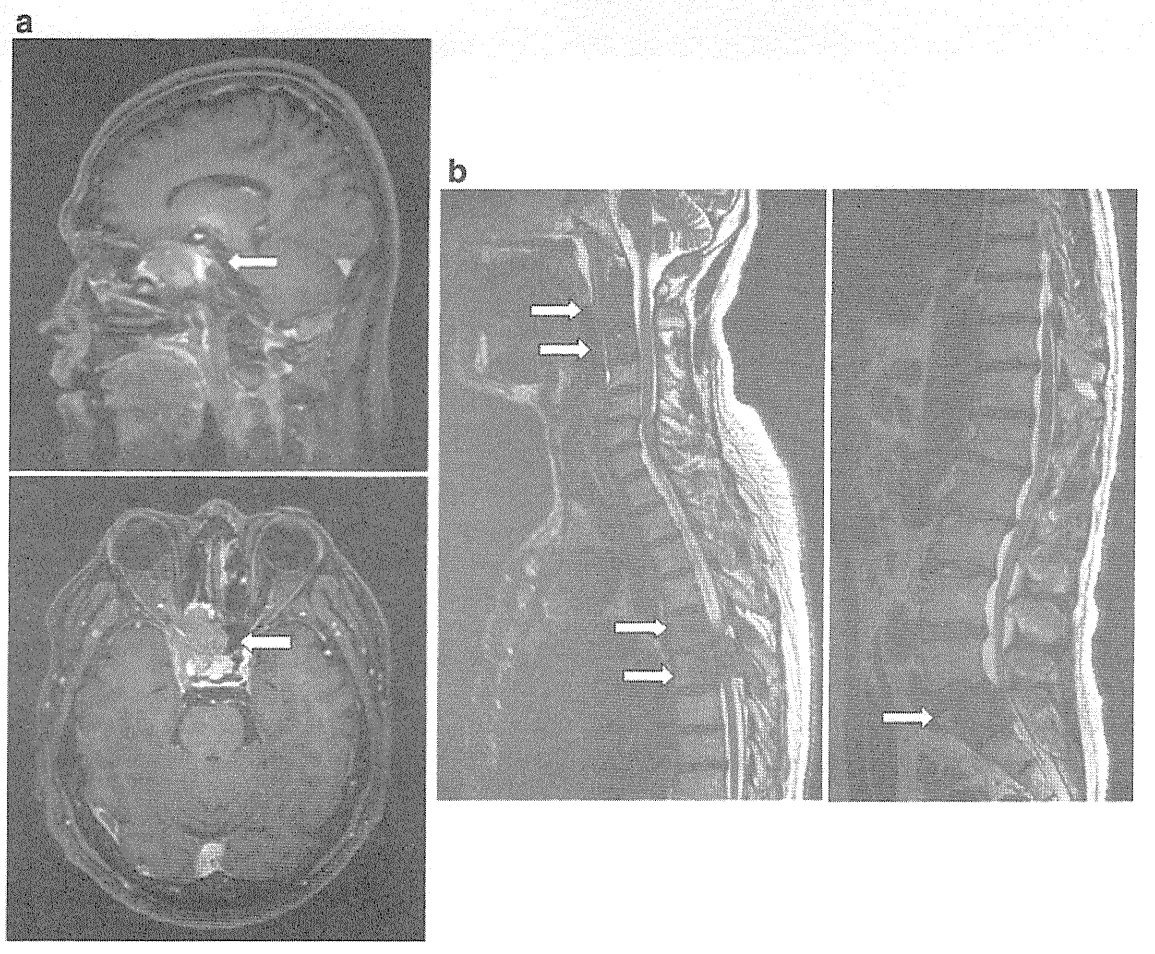

Fig. 2 Cranial and spinal magnetic resonance imaging (*MRI*) on admission. **a** Enhanced cranial MRI, T1-weighted image. **b** Spinal MRI, T2-weighted image. *Arrows* indicate the mass in each image

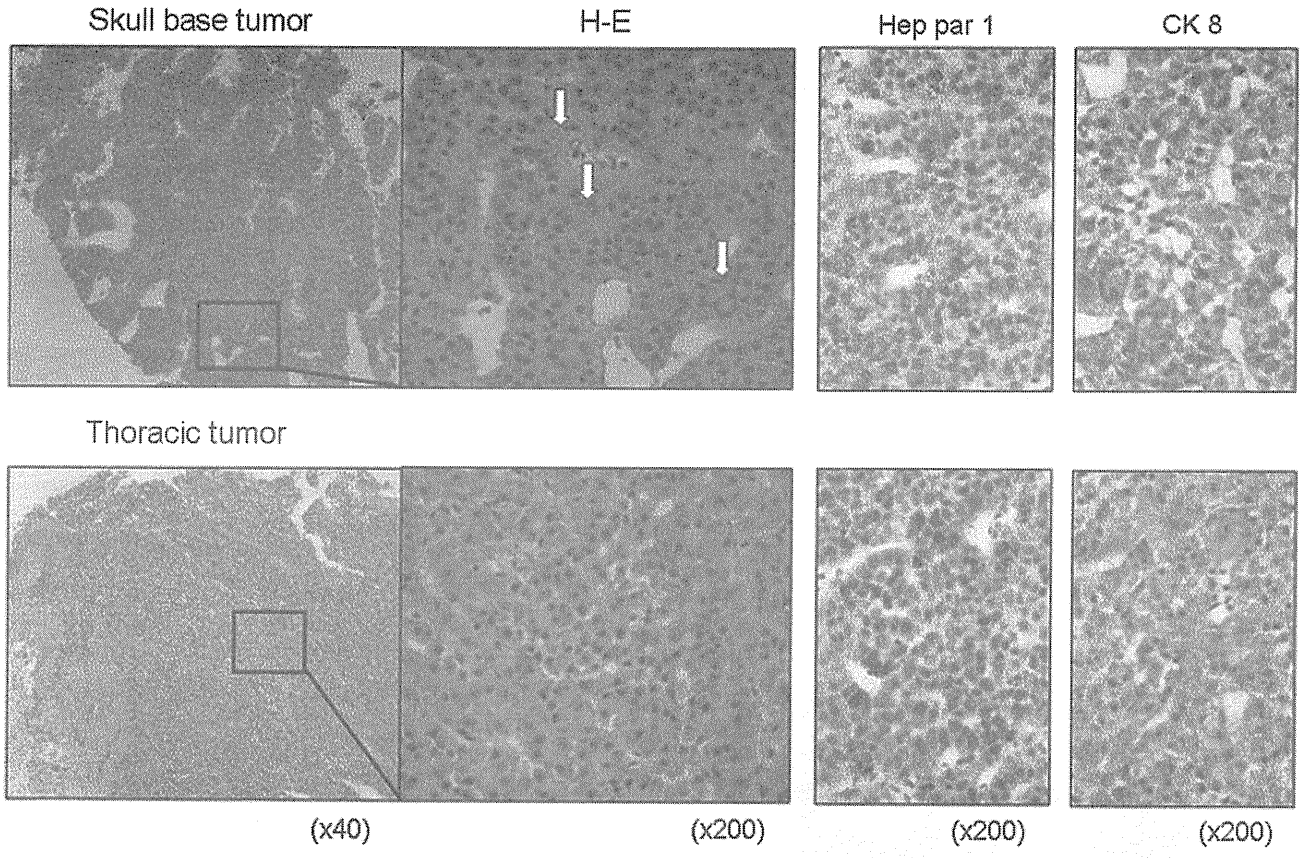

Fig. 3 Microscopic findings. *Upper panel* shows the specimen of the skull base tumor obtained by endoscopic transnasal tumor biopsy. *Lower panel* shows the specimen of the thoracic tumor obtained by laminectomy. Hematoxylin and eosin (H&E), HepPar1, and cytokeratin (CK) 8 antibodies were used for immunocytochemical study. *Arrows* indicate bile pigments

on day 1) and cisplation (100 mg on day 1) (FMP) therapy [10]. However, the general condition of the patient deteriorated and he died 5 months after admission to our hospital.

Discussion

HCC is known as a very aggressive cancer causing intrahepatic metastases via portal vein and hepatic veins at early stages, while distant metastases usually occur at late stages, possibly via hematogenous or lymphatic pathways [1]. Therefore, it is important to find the primary HCC in the liver at an early stage for a better prognosis for the patient. However, HCC is also known to be a cancer characterized by poor presentation of specific symptoms until advanced stages. Thus, the symptoms due to the metastasis sometimes become the first presentation of the disease and provide an opportunity to find the primary HCC, as in our case.

Some reports have described the most common clinical presentations of cranial HCC to be scalp mass, neurological deficits, headache and seizures [3, 11, 12]. Theoretically, the clinical symptoms should be associated with the cranial site of the tumor involved, i.e., scalp mass and headache are likely due to calvarial metastases, and cranial nerve deficits are likely due to skull base metastases. In this report, the skull base tumor of the patient located as involving only the optic canal may have disturbed the optic nerve in preference to the other cranial nerves. When we focus on the symptom of visual disturbance, the most common previously reported visual disturbances from HCC are homonymous hemianopia, diplopia, and proptosis, which are mostly due to orbit metastasis [13–16]. Blindness is a very rare symptom of metastatic tumors derived from the trunk of the body, like HCC, gastric cancer [17], and esophageal cancer [18]. By a Medline search via PubMed, HCC is reported in one case as presentating with an occipital haematoma after therapy for metastatic HCC [19]. To the best of our knowledge, blindness has not been reported as the initial presentation for metastatic HCC.

Hepatitis C virus (HCV) and HBV infection are well known major causes of HCC, however, the incidence of HCC is lower in alcoholic cirrhosis in the absence of HCV and HBV infection [20]. In this paper, the laboratory

investigation of the patient revealed negative hepatitis B surface antigen, however, HBV-DNA PCR was substantively positive. We examined HBV-DNA PCR three times and the results were all positive: 2.5, 2.5, and 2.1 log copy/ml. Occult HBV infection is defined as serologically undetectable hepatitis B surface antigen despite the presence of circulating HBV-DNA, suggesting maintaining a potential risk for HCC development [21]. Thus, the liver dysfunction of this patient might be due not only to alcohol abuse but also to 'occult hepatitis B' infection, and the synergistic effect of this combination might be the main cause of HCC.

A strict diagnosis of metastatic HCC is usually difficult by noninvasive examination, because CT and MR images of metastatic HCC vary a great deal in each case [3]. Even though the specific tumor markers for HCC of our patient were high (AFP 1881 ng/ml; PIVKA-II 6911 mAU/ml), a histological diagnosis was necessary for the identification of primary cancer. Thus, we performed an immunocytochemical study of the specimen obtained by endoscopic transnasal tumor biopsy and laminectomy (Fig. 3). The result of the immunochemical expression of the effective markers for HCC, HepPar1 and CK 8 antibodies, suggested that the metastatic tumors were derived from hepatocyte [7–9]; both tumors were strictly diagnosed as metastasis of moderately differentiated HCC.

In conclusion, although blindness is an exceedingly rare symptom of HCC, it is necessary to be aware of it. To the best of our knowledge, this is the first report of a HCC patient with monocular blindness as the initial presentation of the disease.

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＜短　報＞

B型肝炎ウイルスジェノタイプB型感染高浸淫地区における感染実態の変遷

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芳賀　弘明[1)]　奥本　和夫[1)]　西瀬　雄子[1)]　河田　純男[2)]

はじめに：わが国において，B型肝炎ウイルス（HBV）感染者のHBVジェノタイプは，全体の80%以上を占めるC型がmajor typeであるが，その分布には地域特性が知られている．以前我々は，全国多施設共同研究の一環として，当科で診療したHBs抗原陽性者についてジェノタイプ調査をretrospectiveに行ったが，ジェノタイプB型が34.6%を占めていた[1)]．

また，HBVジェノタイプA型感染によるB型急性肝炎は都市部においてその拡がりが顕著であるが[2)3)]，地方においても増加傾向にあるものと推測され，今後の感染拡大が予想される．また肝炎の遷延化も指摘されており[4)]，ユニバーサルワクチン導入の是非を議論する上でも，その感染実態を把握することは重要である．HBVジェノタイプB型高浸淫地域における，最近のB型急性肝炎のジェノタイプによる感染実態は明らかではない．そこで，過去20年間における，当科のHBs抗原陽性例のHBVジェノタイプ別感染の変遷，およびB型急性肝炎におけるHBVジェノタイプの感染実態の変遷を検討した．

対象および方法：1990年から2009年まで，当科で診療したHBs抗原陽性全例にあたる430例を解析対象とした．これらのHBVジェノタイプを測定し，1990年から1999年（284例），2000年から2009年（146例）の二群に分けて，各HBVジェノタイプ別の感染頻度の変遷を検討した．HBVジェノタイプは当該期間内に初回受診した際の凍結保存血清を用いてretrospectiveに解析した．また430例の中で血清学的にB型急性肝炎と診断された34例について同様の検討を行った．B型急性肝炎の診断は，1）血清HBs抗原陽性かつIgM-HBc抗体陽性であること，2）他の肝炎の原因が血清学的に否定されていること，3）急性の肝機能障害を認め，過去に肝機能障害の既往がないこと，とした．

結果：HBs抗原陽性430例におけるジェノタイプ別のHBV感染者の割合は，A型11例（2.6%），B型194例（45.0%），C型150例（34.9%），D型4例（1.0%），分類あるいは検出不能による不明71例（16.5%）であった．1999年以前では，A型6例（2.1%），B型128例（45.1%），C型100例（35.2%）であったのに対し，2000年以降では，A型5例（3.5%），B型66例（45.2%），C型50例（34.2%）であった．いずれの年代においても，HBs抗原陽性例全体におけるジェノタイプの感染実態には変化は見られなかった．

B型急性肝炎34例のジェノタイプ別のHBV感染者の割合は，1999年以前の群に比較し2000年以降の群ではジェノタイプB型の割合が有意に低下し（10/17；58.8% vs. 1/17；5.9%：$p=0.012$），ジェノタイプA型の割合は増加傾向であった（2/17；11.8% vs. 5/17；29.4%，Fig.）．

当科で経過観察されているHBVジェノタイプA型感染によるB型急性肝炎症例7例を示す（Table）．ジェノタイプはすべてAe型であった．感染経路は，患者本人からの問診によればいずれも異性間性行為が疑われ，同性愛者はいなかった．また推定される感染地域は，不明である症例3，6を除けばいずれも山形県内であった．症例1は他の6例と異なり，自覚症状がなく，職場検診で初めてALT値の上昇とHBs抗原陽性を指摘され，IgM-HBc抗体陽性であったことから，B型急性肝炎と診断された．ラミブジンが投与されたが改善せず，その後ラミブジン耐性ウイルスが出現し，アデフォビルの投与によりALT値とウイルス量の低下を見た．肝機能異常を検診で初めて指摘されてから約3カ月後のラミブジン投与前の肝生検では，既に慢性肝炎（F1/A1）の組織像を呈していた．他の6例は，来院時にいずれも急性肝炎に特徴的な症状とALT値の高値を伴い，その後HBs抗原の陰性化を確認している．

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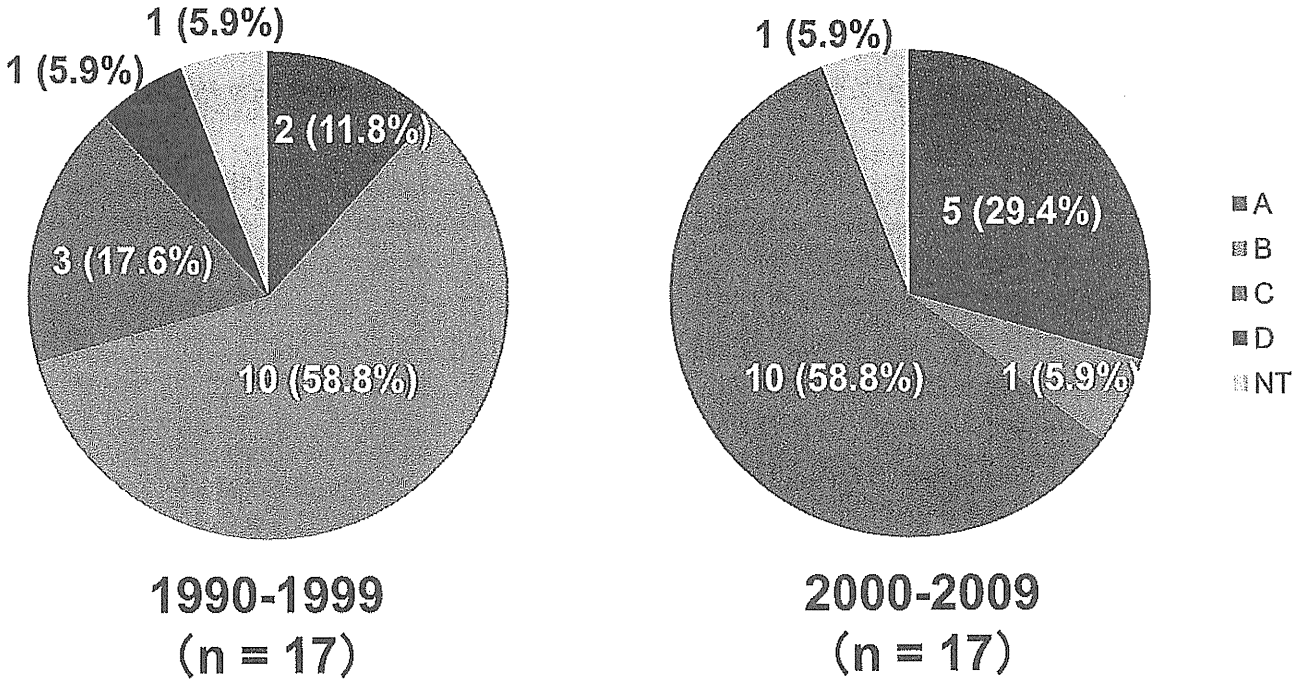

Fig. Distribution of HBV genotypes in acute hepatitis B
Genotype B was found in 58.8% between 1990 and 1999, 5.9% between 2000 and 2009, while the prevalence of genotype A was increased from 11.8% (1990-1999) to 29.4% (2000-2009).

Table Characteristics of 7 patients infected with genotype A

Case No.	Age (Years)	Sex	ALT* (IU)	IgM anti-HBc (S/CO)	HIV Ab	HBsAg clearance (months)	Outcome	Treatment	Route of Infection	Genotype
1	45	M	185	10.2	(−)	Persistently positive	chronicity	LAM	heterosexual	Ae
2	22	M	1282	41.3	(−)	4	resolved	ETV	heterosexual	Ae
3	26	M	2008	26.3	(−)	9	resolved	ETV	heterosexual	Ae
4	49	F	557	22.6	(−)	5	resolved	—	heterosexual	Ae
5	26	M	3650	29.9	(−)	3	resolved	—	heterosexual	Ae
6	56	M	2876	8.3	(−)	3	resolved	—	heterosexual	Ae
7	55	F	1414	7.2	(−)	5	resolved	—	heterosexual	Ae

LAM: Lamivudine, ETV: Entecavir
*the first medical examination on admission

考察：HBV ジェノタイプB型高浸淫地域において，HBs 抗原陽性例全体に占める HBV ジェノタイプを検討した結果，ジェノタイプB型およびC型の感染割合は過去と現在においてほとんど変化していないことが明らかとなった．

また過去20年における34例のB型急性肝炎の HBV ジェノタイプの検討では，1999年以前にはジェノタイプB型感染者が半数を占めていたが，最近10年間ではわずかに1/17例とほとんど見られなくなっていた．従来 HBV ジェノタイプB型感染者が圧倒的に多い当施設において，ジェノタイプB型感染のB型急性肝炎例の割合が2000年以降に急激に低下していることは興味深い．一方，急性肝炎に占めるジェノタイプC型感染の割合はこの10年間で増加していたが，その理由としてジェノタイプA型感染の増加と相まって，本州における主なジェノタイプであるC型感染も地方へ広がって

きているためと推測する．

本研究では必ずしも一般の急性肝炎を反映しているとは言えないが，少なくとも過去にジェノタイプB型感染の頻度が高かった単一施設での観察では，ジェノタイプB型感染が減少し，ジェノタイプA型感染が確実に増加してきていることが明らかとなった．特に急性期に自覚症状を欠き検診などで偶然に見つかる例にも遭遇することから，地方におけるジェノタイプA型感染の拡大にも，今後は十分に留意する必要があるものと思われた．

結語：HBVジェノタイプB型感染の高浸淫地域において，この20年間で，HBs抗原陽性例全体に占めるジェノタイプB型感染の比率に変化はないものの，B型急性肝炎のジェノタイプの感染割合は，ジェノタイプB型が明らかに減少し，ジェノタイプA型が増加していた．肝炎の遷延化の問題も含め，全国的な疫学調査に基づいたB型肝炎対策が急務である．

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索引用語：HBV genotype，acute hepatitis，genotype B

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英文要旨

Hepatitis B virus genotypes in a hyperendemic area for genotype B infection

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To elucidate genotypes of HBV carriers in a hyperendemic area for HBV genotype B infection and to examine the changes over time in genotypes responsible for acute hepatitis B, 430 HBsAg-positive HBV carriers were determined by genotypes and compared the literal translation of infection status according to two time-period groups: a group seen between 1990 and 1999 and a group seen between 2000 and 2009.

In total, 45% had genotype B and 35% had genotype C in both time-period groups, indicating no changes in genotypes over time. Among 34 acute hepatitis B patients, the percentage of genotype B was significantly lower in the present group (5.9%) than in the past group (58.8%), while the prevalence of genotype A tended to have increased in the last 10 years.

In conclusion, there was an increase in acute hepatitis B infection by genotype A in a hyperendemic area for genotype B infection, even though there was no large change of genotypes between the present and the past percentages of subjects. A nation-wide surveillance of HBV infection status is a matter of urgency in terms of the universal vaccination for HBV.

Key words: HBV genotype, acute hepatitis, genotype B

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Research Paper

Efficacy of Lamivudine or Entecavir on Acute Exacerbation of Chronic Hepatitis B

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Abstract

Background/Aims: Spontaneous acute exacerbation of chronic hepatitis B virus (HBV) infection occasionally occurs in its natural history, sometimes leading rapidly to fatal hepatic failure. We compared the effects of lamivudine (LAM) with those of entecavir (ETV) treatments in acute exacerbation of chronic hepatitis B with 500 IU/L or higher alanine aminotransferase (ALT) levels.

Methods: Thirty-four patients with acute exacerbation were consecutively treated with LAM /ETV. Their clinical improvements were compared.

Results: Among LAM-treated and ETV-treated patients, none showed a reduction of <1 log IU/mL in HBV DNA after 1 or 3 months of treatment. Initial virological response, defined as a reduction of 4 log IU/mL in HBV DNA at 6 months, with LAM and ETV, respectively, was 83.3% and 100%. One LAM patient developed hepatic encephalopathy, but all patients in both groups survived. Twelve months after treatment, 41.6% of 24 LAM group patients switched to another drug or added adefovir to their treatment due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change drugs.

Conclusions: ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should carefully start to treat these patients as soon as possible.

Key words: acute exacerbation, ALT, entecavir, HBV, lamivudine

INTRODUCTION

Chronic hepatitis B infection is associated with the development of hepatocellular carcinoma [1]. Infection with hepatitis B virus (HBV) also leads to wide a spectrum of liver injury, including acute, self-limited infection, fulminant hepatitis, and chronic hepatitis with progression to cirrhosis and liver failure, as well as to an asymptomatic chronic carrier state [2, 3].

Reactivation of hepatitis B is a well-characterized syndrome marked by the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactivated or resolved HBV infection [4]. Reac-

tivation is often spontaneous, but can also be triggered by cancer chemotherapy and immune suppression. Spontaneous acute exacerbation of chronic hepatitis B infection is seen with a cumulative probability of 15-37% after 4 years of follow-up [5]. Prognosis is generally poor in HBV carriers with spontaneous acute exacerbation together with high alanine aminotransferase (ALT) levels, jaundice, and liver failure [4, 6, 7]. This condition has been defined as acute-on-chronic liver failure according to a recent Asia-Pacific consensus recommendation [8]. Acute exacerbation occasionally leads to a critical scenario, meaning that clinicians need to treat this condition immediately.

Lamivudine (LAM) is a reverse-transcriptase inhibitor of viral DNA polymerase with an excellent profile of safety and tolerability, causing inhibition of viral replication, and it is approved for antiviral treatment of hepatitis B patients [9, 10]. LAM suppresses serum HBV DNA values in up to 98% of patients within a median period of 4 weeks, leading to aminotransferase normalization, increased hepatitis B e antigen (HBeAg) seroconversion rate, and improvement of histological parameters [11, 12]. A study from Taiwan showed that LAM had a survival benefit and was effective for patients with baseline bilirubin levels below 20 mg/dL [7].

Entecavir (ETV), a deoxyguanosine analogue, is a potent and selective inhibitor of HBV replication; its *in vitro* potency is 100- to 1,000-fold greater than that of LAM, and it has a selectivity index (concentration of drug reducing the viable cell number by 50% [CC_{50}]/concentration of drug reducing viral replication by 50% [EC_{50}]) of ~8,000 [13, 14]. At present, the Japanese national health insurance system approves ETV as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is a nucleoside analogue (NUC) belonging to a new subgroup, cyclopentane [15], and it has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing ALT, although NUCs do not eradicate the virus. ETV develops less resistance than LAM.

We undertook a retrospective study to compare the efficacy of LAM with that of ETV in the reduction of HBV DNA levels and associated improvement in disease severity and biochemical recovery in patients with acute exacerbation together with higher ALT levels due to HBV reactivation.

MATERIALS AND METHODS

Patients

A retrospective analysis of LAM/ETV-treated chronic hepatitis B patients at Chiba University Hospital and Numazu City Hospital, Japan, between May 2003 and December 2009 was performed. The inclusion criteria were: acute exacerbation of chronic hepatitis B characterized by an elevation of ALT level ≥ 500 IU/L along with HBV DNA ≥ 4.5 log IU/mL presenting in a patient with diagnosed chronic liver disease. The exclusion criteria were: acute hepatitis B, superinfection with other viruses (hepatitis E, A, D, or C), other causes of chronic liver failure [16, 17], coexistent hepatocellular carcinoma, portal thrombosis, coexistent renal impairment, pregnancy, coinfection with human immunodeficiency virus (HIV), or patients who had received a previous course of NUC treatment. This retrospective study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of Chiba University, Graduate School of Medicine [18].

Baseline assessment of patients

Retrospectively collected data included patient demographics, clinical findings, all laboratory variables including virological tests and abdominal ultrasound. HBsAg, HBeAg, anti-HBe antibody and immunoglobulin M (IgM) anti-HBc antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [19]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [20]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan).

Definitions

Primary antiviral treatment failure was defined as a reduction of < 1 log IU/mL in HBV DNA after 3 months of therapy. Initial virological response (IVR) was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

Follow-up

Clinical assessment and routine investigations were done every 15 days or every month for at least 6 months. HBV DNA measurements were repeated monthly.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7 and StatView 5 (SAS Institute Inc, Cary, NC). Continuous variables were expressed as mean ± standard deviation and were compared by two-factor analysis of variance (ANOVA) and two-way repeated measures ANOVA. Categorical variables were compared by Chi-square

test. Baseline was taken as the date when the first dose of LAM/ETV was administered. Statistical significance was considered at a *P*-value < 0.05.

RESULTS

Patients

Between May 2003 and December 2009, 34 patients with spontaneous acute exacerbation of chronic hepatitis B, with ALT levels ≥ 500 IU/mL and treated with LAM or ETV, were consecutively enrolled and retrospectively analyzed. 24 (70.5%) were treated with LAM at 100 mg daily and 10 (29.4%) were treated with ETV at 0.5 mg daily. All patients were followed for at least 6 months. Mean follow-up in the LAM and ETV groups was 55.5 ± 25.4 and 16.5 ± 9.9 months, respectively.

Baseline characteristics

Baseline characteristics in the two patient groups were similar **(Table 1)**. Median age was 37 (21-73) years and 79.4% were men. One patient of the LAM group developed hepatic encephalopathy, but recovered. All patients in both groups survived. At admission, the serological profile showed HBsAg positivity in all 34 (100%); 22 (64.7%) were HBeAg positive. The median HBV DNA level was 7.4 log IU/mL in the LAM group and 7.9 log IU/mL in the ETV group **(Table 1)**.

Table 1 Demographic, Clinical, and Laboratory Variables of Patients at Entry.

Parameters	Total Patients (N=34)	LAM (N=24)	ETV (N=10)	*P*-value
Age (years)	37 (21-73)	37 (21-73)	39 (24-67)	NS
Male (%)	27 (79.4)	18 (75)	9 (90)	NS
Cirrhosis (+/-)	2/32	2/22	0/10	NS
ALT (IU/L)	986 (523-2,450)	995 (523-2,450)	1,046 (523-2,140)	NS
T. Bil (mg/dL)	2.0 (0.8-22.0)	2.4 (0.8-20.6)	1.6 (1.9-22.0)	NS
PT (%)	83 (24-121)	81.5 (24-119)	83.6 (35-121)	NS
HBeAg (+/-)	22/12	18/6	4/6	NS
HBV DNA (log IU/mL)	7.6 (4.8-8.7)	7.4 (5.2-8.7)	7.9 (4.8-8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in HBV DNA of total patients

LAM significantly reduced HBV DNA levels from baseline 7.24 log IU/mL to 3.27 log IU/mL at 1 month ($P < 0.001$), to 2.21 log IU/mL at 3 months ($P < 0.001$), and to 1.53 log IU/mL at 6 months ($P < 0.001$). ETV also significantly reduced HBV DNA levels from baseline 7.56 log IU/mL to 3.12 log IU/mL at 1 month ($P < 0.001$), to 2.14 log IU/mL at 3 months ($P < 0.001$), and to 1.77 log IU/mL at 6 months ($P < 0.001$). There were no differences in HBV DNA levels from baseline to 6 months between the two groups. None with primary antiviral treatment failure was identified in either group. There were no significant differences in IVR between the two groups **(Figure 1)**.

Reduction in ALT levels of total patients

LAM significantly reduced ALT levels from baseline 1,130 IU/mL to 102 ($P < 0.001$) at 1 month, to 28.6 ($P < 0.001$) at 3 months, and to 23.1 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,210 IU/mL to 117 ($P < 0.001$) at 1 month, to 25 ($P < 0.001$) at 3 months, and to 24.4 ($P < 0.001$) at 6 months. There were no differences in ALT levels from baseline to 6 months between the two groups **(Figure 2)**.

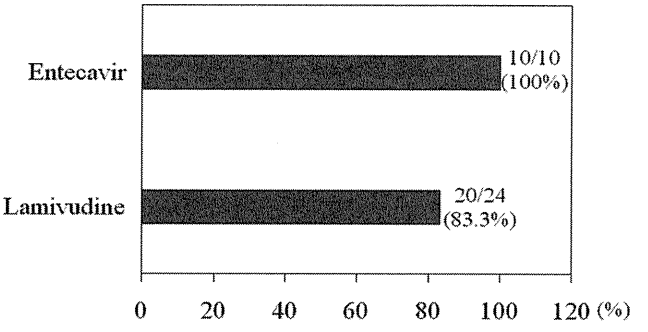

Figure 1 Initial virological response (IVR). IVR was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

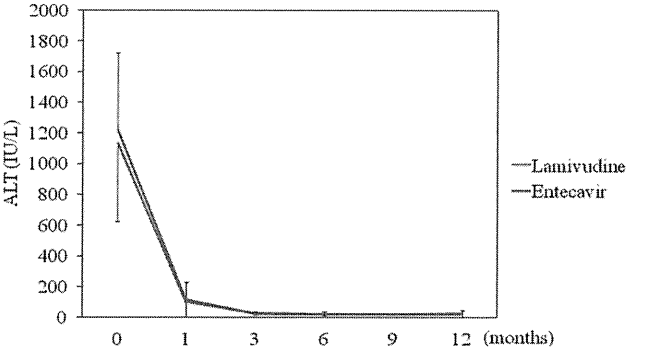

Figure 2 Efficacy of lamivudine and entecavir for ALT levels. Lamivudine (N=24) vs. entecavir (N=10); data are shown as mean ± SD.

Reduction in HBV DNA of HBeAg-positive patients

It has been demonstrated that the levels of HBV DNA in the HBeAg-positive phase were generally higher than those in the ant-HBe-positive phase [19, 22]. HBeAg positivity is also associated with HBV viremia and increased ALT levels in HIV/HBV co-infected patients [23]. Next, we compared the response to LAM or ETV in 18 or 4 HBeAg-positive patients, respectively **(Table 2)**. LAM significantly reduced HBV DNA levels from baseline 7.52 log IU/mL to 3.35 log IU/mL ($P < 0.001$) at 1 month, to 2.38 log IU/mL ($P < 0.001$) at 3 months, and to 1.55 log IU/mL ($P < 0.001$) at 6 months. ETV also significantly reduced HBV DNA levels from baseline 8.42 log IU/mL to 3.87 log IU/mL ($P < 0.001$) at 1 month, to 2.90 log IU/mL ($P < 0.001$) at 3 months, and to 2.22 log IU/mL ($P < 0.001$) at 6 months. There were no differences in HBV DNA levels from baseline to 6 months between the two groups. Primary antiviral treatment failure was not observed in either group. Four patients in the LAM group did not achieve IVR.

Table 2 Demographic, Clinical, and Laboratory Variables of HBeAg-positive Patients at Entry.

Parameters	Total Patients (N=22)	LAM (N=18)	ETV (N=4)	*P*-value
Age (years)	34.5 (21-51)	36.5 (21-51)	30 (24-33)	NS
Male (%)	18 (81.8)	14 (77.7)	4 (100)	NS
Cirrhosis (+/-)	1/21	1/17	0/4	NS
ALT (IU/L)	1,030 (523-2,450)	1,990 (523-2,450)	1,363 (980-1,620)	NS
T. Bil (mg/dL)	1.75 (0.8-20.6)	2.0 (0.8-20.6)	1.5 (1.0-18.7)	NS
PT (%)	77 (24-119)	73.6 (24-119)	95.0 (44.1-113)	NS
HBeAg (+)	22	18	4	
HBV DNA (log IU/mL)	7.6 (5.5- 8.8)	7.6 (5.5- 8.7)	8.6 (7.6- 8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in ALT levels of HBeAg-positive patients

LAM significantly reduced ALT levels from baseline 1,150 IU/mL to 84 ($P < 0.001$) at 1 month, to 27.5 ($P < 0.001$) at 3 months, and to 22.0 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,460 IU/mL to 230 ($P = 0.0038$) at 1 month, to 22.2 ($P = 0.0016$) at 3 months, and to 24.0 ($P = 0.0016$) at 6 months. At 1 month after treatment, the ALT levels of the LAM groups were lower than those of the ETV group ($P < 0.0001$) **(Figure 3)**. During follow-up periods, 10 and 1 sero-converters of HBeAg to anti-HBe antibody phase were seen in 18 LAM-treated and in 4 ETV-treated patients, respectively.

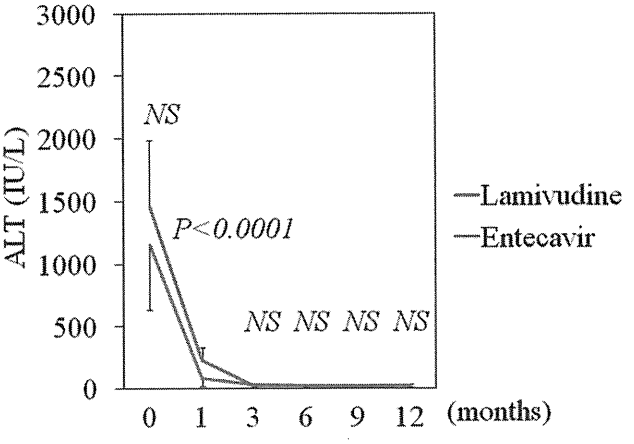

Figure 3 Efficacy of lamivudine and entecavir for ALT levels in HBeAg-positive patients. Lamivudine (N=18) vs. entecavir (N=4); data are shown as mean ± SD.

Safety

No patient stopped taking medications. Twelve months after treatment, 10 of 24 patients (41.6%) in the LAM group switched from LAM to ETV (n=4) or added adefovir (n=6) due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change their medication.

DISCUSSION

The present study compared the use of NUCs, LAM and ETV, for the treatment of acute exacerbation of chronic hepatitis B. The results clearly showed significant benefits of a rapid reduction of HBV DNA levels, compared with untreated patients in a previous report [4].

It was reported that ETV treatment is associated with increased short-term mortality in patients with severe acute exacerbation of chronic hepatitis B, but that it achieves better virological response in the long run [24]. We used LAM or ETV for patients with acute exacerbation of chronic hepatitis B presenting with ALT ≥ 500 IU/L in the present study. The effects of LAM on HBV DNA levels were the same as those of ETV (Figure 1). But the effects of LAM on ALT levels after 1 month were stronger than those of ETV in HBeAg-positive patients **(Figure 3)**. In spite of the limited number of these patients, the effects were possibly related to immunomodulating activities of LAM [25]. The patients' prognoses were more favorable than in the previous report [4]. This might have

depended on the fact that, in the present study, treatment was begun as soon as possible, and some patients may have had a milder grade of acute exacerbation of chronic hepatitis B than those in the previous report [4]. We believe that patients with acute exacerbation of chronic hepatitis B need to be subjected to treatment as promptly as possible.

The major routes of HBV infection in our country have been mother-to-child transmission and blood transfusion. However, cases with HBV transmitted through sexual contact are increasing, especially among HIV-1-seropositive patients [26]. One should bear in mind that knowledge about interactions between ETV and anti-HIV nucleoside analogues is limited [27]. Because long-term use of LAM induces LAM-resistant mutants [28], we can only use LAM for short-term treatment of patients with acute exacerbation of chronic hepatitis B. On the other hand, the present study also revealed that patients receiving ETV did not need to change drugs.

Recently, there have been several reports that reactivation of HBV is a fatal complication following systemic chemotherapy or other immunosuppressive therapy including rituximab and steroid therapies mainly in HBsAg-positive and -negative lymphoma patients. It is important to enable early diagnosis of HBV reactivation as well as initiation of antiviral therapy [29, 30].

In conclusion, ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should start to treat these patients with NUCs as soon as possible.

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ABBREVIATIONS

ETV: Entecavir; HIV: Human immunodeficiency virus; IVR: Initial virological response; LAM: Lamivudine; NUC: Nucleoside analogue.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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Clinical importance of serum hepatitis B surface antigen levels in chronic hepatitis B

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SUMMARY. Quantitative serology for hepatitis B surface antigen (HBsAg) is a new candidate marker for prediction of clinical outcome. The aim of this study was to investigate the clinical significance of quantifying HBsAg in patients with hepatitis B virus (HBV) infection. A total of 424 patients who tested positive for HBsAg and were referred to Chiba University Hospital between January 1985 and April 2008 were included in the study, and the following characteristics were analyzed: age, gender, status of hepatitis B e antigen (HBeAg), alanine aminotransferase level (ALT), HBV DNA level, number of platelets and development of hepatocellular carcinoma. Measurement of HBsAg was performed using the chemiluminescent enzyme immunoassay method. The study group consisted of 239 men and 185 women, and their average age was 40.6 ± 14.0 years. HBsAg showed a positive correlation with HBV DNA level (Pearson's product moment correlation, $r = 0.586$, $P < 0.001$) and a weak inverse correlation with age ($r = 0.3325$, $P < 0.001$). A control study, matched with age and sex, was performed between two groups with and without HBeAg seroconversion during follow-up period. Compared with the age and sex-matched controls, the change in HBsAg levels per year showed a significant decrease 2 years before seroconversion (paired *t*-test, $P < 0.05$). The serial measurement of quantitative HBsAg level has the possibility of predicting the occurrence of HBeAg seroconversion.

Keywords: chronic hepatitis B, HBeAg seroconversion, HBs antigen quantification.

INTRODUCTION

An estimated 350 million persons worldwide are chronically infected with HBV [1]. Chronic infection with HBV can progress to cirrhosis, liver failure and hepatocellular carcinoma (HCC), and is a major cause of mortality worldwide [2,3]. Loss of hepatitis B e antigen (HBeAg), accompanied by seroconversion to anti-HBe antibody, usually results in normalized serum alanine aminotransferase (ALT) and decreased HBV DNA levels, and may lead to improved hepatic necroinflammation and confer a better clinical outcome [4–6]. In a recent study of the natural history of chronic hepatitis B (CHB) in 3233 Asian patients, the median age of HBeAg seroconversion was 35 years [7]. HBeAg seroconversion may occur spontaneously at a rate of 5–10% per year [8]. Thus, in clinical practice, HBeAg seroconversion is recognized as a successful serologic response to the treatment of HBeAg-positive CHB.

Determining an accurate prognosis for HBV carriers, based on clinical presentation, is important for clinical management of the disease. Various studies have been performed to distinguish the positive and negative prognostic factors for HBV carriers. The level of HBV DNA, evaluated by TaqMan® PCR method, is an important predictor of clinical outcome in patients with HBV infection [9], but its efficacy is limited [10]. Therefore, we need another marker for predicting the clinical outcome of HBV carriers. Recently, quantitative serology for hepatitis B surface antigen (HBsAg) has been developed as one of the promising candidates. Chan *et al.* [11] found that peginterferon (PegIFN) alfa-2a provided a significant reduction in HBsAg level in the sera of patients with HBeAg-positive CHB. Moreover, HBsAg decline was significantly associated with HBeAg seroconversion

Abbreviations: ALT, serum alanine aminotransferase; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CI, confidence interval; CLEIA, chemiluminescent enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; HBcrAg, hepatitis B virus core-related antigens; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBV DNA, hepatitis B virus deoxyribo nucleic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IU, international units; LC, log copies; NA, nucleoside/nucleotide analogues; OR, odds ratio; PCR, polymerase chain reaction; PegIFN, peginterferon; PLTs, number of platelets; SD, standard deviation.

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1 year post-treatment, and on-treatment HBsAg levels could be used as an early predictor of durable off-treatment response to PegIFN-based therapy in the individual patient. Recently, Chan *et al.* [12] reported about HBsAg reduction and the fluctuation in titre before and after HBeAg seroconversion of untreated patients.

In this study, based on a cohort of patients with CHB with long-term follow-up, we investigated the HBsAg levels at various stages of CHB. We also aimed to investigate the value of quantitative HBsAg for predicting clinical outcomes in HBeAg-positive CHB patients. Our results clarified the importance of evaluating serum HBsAg levels in patients with CHB.

MATERIAL AND METHODS

Patients

This was a retrospective analysis. Between January 1985 and April 2008, all patients visiting the Chiba University Hospital and who were HBsAg-positive carriers (n = 676) were approached for participation in the study. This study was reviewed and approved by the institutional review board of Chiba University School of Medicine. The patients' consent was obtained for the storage and use of serum. Patients who were positive for hepatitis C virus antibody and those who had another potential cause of chronic liver diseases (autoimmune hepatitis and primary biliary cirrhosis) were excluded from the study. Those patients with <1 year of observation or who had been given antiviral drugs (lamivudine or entecavir) at entry also were excluded from the analysis. As a result, 424 patients were selected for further analysis. To clarify the relationship between the level of HBsAg and other factors, HBV DNA, alanine aminotransferase (ALT) and the number of platelets (PLTs) were analyzed. In addition, we analyzed whether the level of HBsAg was related to the occurrence of HCC. The serum samples from the patients were stored at −20 °C, and the oldest sample obtained from each patient was used to define the level of HBV DNA and HBsAg at entry.

Laboratory assays

Measurement of HBsAg was performed using the chemiluminescent enzyme immunoassay (CLEIA) method and the HISCL-2000i (Sysmex Corporation, Kobe, Japan). HBeAg and anti-HBe levels were determined by enzyme-linked immunosorbent assay (ELISA; Abbott Laboratory, Chicago, IL, USA). Anti-HCV was detected by ELISA (Ortho Diagnostics, Tokyo, Japan). The serum HBV DNA level was quantified by polymerase chain reaction (PCR) assay (Amplicor HBV Monitor; Roche Diagnostics, Basel, Switzerland) with a linear range of quantification of 2.6–7.6 log copies (LC) per mL. The six major genotypes of HBV (A–F) were determined by ELISA (HBV Genotype EIA; Institute of Immunology Co., Ltd., Tokyo, Japan). HBV serum core–related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan).

Serial changes in HBsAg levels during long-term follow-up of HBeAg-positive patients

To observe the serial changes in the HBsAg levels in HBeAg-positive patients, we extracted the HBeAg-positive patients at the beginning of the observation period. Among 424 HBsAg-positive patients, 183 were HBeAg positive. To clarify the long natural history of HBV carriers, we excluded those who could not be followed for more than 5 years. Finally, 120 patients who could be followed for more than 5 years were enrolled and their HBsAg levels were evaluated every year with an error of <2 months.

Statistical analysis

The baseline data are presented as mean ± SD or median and range. The difference in the values of clinical parameters between the two groups was analyzed by paired t-test, unpaired t-test, Welch t-test and chi-square test. Pearson's product moment correlation coefficient analysis was used for statistical analyses, as appropriate, with the statistical program SPSS 16.1 (SPSS Inc., Chicago, IL, USA); a P value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics and the relationship between HBsAg quantification and other clinical markers

The baseline clinical and virological characteristics of the 424 HBsAg-positive carriers are shown in Table 1. First, we investigated the relationship between HBsAg and other virological and clinical markers. The relationships of HBsAg (log IU/mL) with age, gender, HBV genotype and HBeAg status are illustrated in Fig. 1. Gender was not associated with HBsAg titre (Fig. 1a). In contrast, the level of HBsAg in the patients with HBV genotype C differed significantly from those with genotype B ($P < 0.05$, unpaired t-test) (Fig. 1b). The average of HBsAg titre was significantly higher in HBeAg-positive patients compared with those who were HBeAg negative, with statistical difference (unpaired t-test, $P < 0.05$, Fig. 1c). HBsAg showed a significant positive correlation with the HBV DNA level (Pearson's product moment correlation, $r = 0.586$, $P < 0.001$, Fig. 2a), and a weak and inverse correlation between HBsAg and age is also shown (Fig. 2b). In contrast, HBsAg did not show a good correlation with ALT level or PLTs (Figs 2c,d). Next, we used the Cox proportional hazards model to investigate whether HBsAg could be a predictive marker for the occurrence of HCC. Screening for the detection of HCC was performed based on the typical findings of abdominal ultrasonography, dynamic computed tomog-

Table 1 Baseline characteristics of HBsAg-positive patients

Parameters	Total patients	HBeAg-positive patients*
Total patients	424	120
Gender (male/female)	239/185	68/52
Age (average ± SD) (years)	40.6 ± 14.0	34.3 ± 13.1
HBeAg status (positive/negative)	183/241	120/0
HBV DNA level (average ± SD) (log copies/mL)	5.5 ± 1.9	7.1 ± 1.2
ALT level (average ± SD) (IU/L)	70.4 ± 79.3	93.2 ± 96.2
PLT number (average ± SD) ($\times 10^4$ number/μL)	20.8 ± 6.6	20.2 ± 6.2
Follow-up (average ± SD) (years)	5.4 ± 5.1	10.0 ± 5.5
Genotype A/B/C/D/not determined	6/30/250/0/138	2/6/110/2
HBsAg level (average ± SD) (log IU/mL)	3.42 ± 1.15	4.02 ± 0.98
Antiviral drugs	48	34
HCC occurrence	18	4

ALT, alanine aminotransferase; PLT, the number of platelets; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; *120 patients of HBeAg-positive patients were followed for more than 5 years.

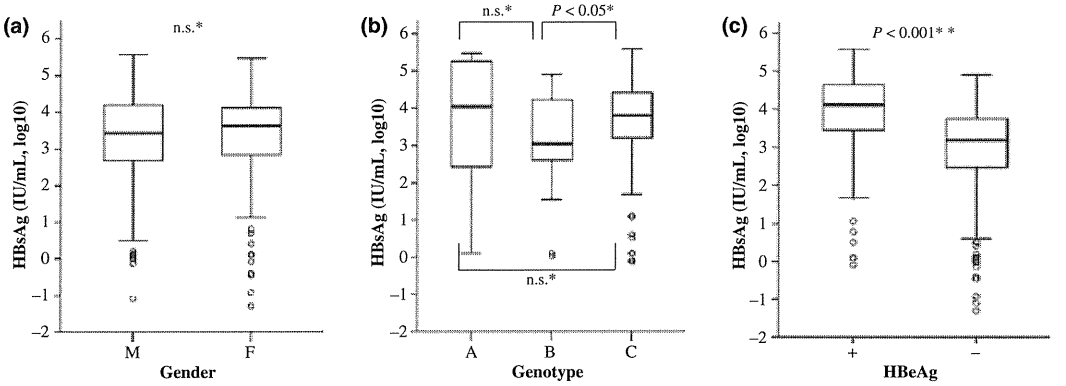

Fig. 1 The association between HBsAg level (log IU/mL) and (a) gender, (b) HBV genotype and (c) HBeAg status. There was no significant difference between HBsAg and gender ($P = 0.146$, unpaired t-test). In contrast, compared with genotype B, the level of HBsAg in the patients with HBV genotype C was significantly different ($P < 0.05$, unpaired t-test). There was a significant difference according to the positive or negative status of HBeAg ($P < 0.001$, unpaired t-test). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

raphy, angiography and/or magnetic resonance imaging. For all of the patients who were suspected as HCC by image analysis, the diagnosis of HCC was confirmed by pathological analysis. Univariate analysis revealed that age [compared with young patients: odds ratio (OR) = 1.10, 95% confidence interval (CI) = 1.01–1.11], number of PLTs (compared with patients of low PLTs: OR = 0.98, 95% CI = 0.97–0.99) and HBV DNA level (compared with patients of low HBV DNA levels: OR = 1.32, 95% CI = 1.05–1.67) at baseline were predictive factors for HCC occurrence, not HBsAg titre (compared with patients of low HBsAg levels: OR = 0.79, 95% CI = 0.56–1.10). Multivariate analysis revealed that age (compared with young patients: OR = 1.07, 95% CI = 1.03–1.11) and number of PLTs (compared with patients of low PLTs: OR = 0.99, 95% CI = 0.98–0.99) at baseline were predictive factors for HCC occurrence.

The effect of serial change of HBsAg in HBeAg-positive HBV carriers

The baseline clinical characteristics of 120 HBeAg-positive carriers are shown in Table 1, and the level of HBsAg were

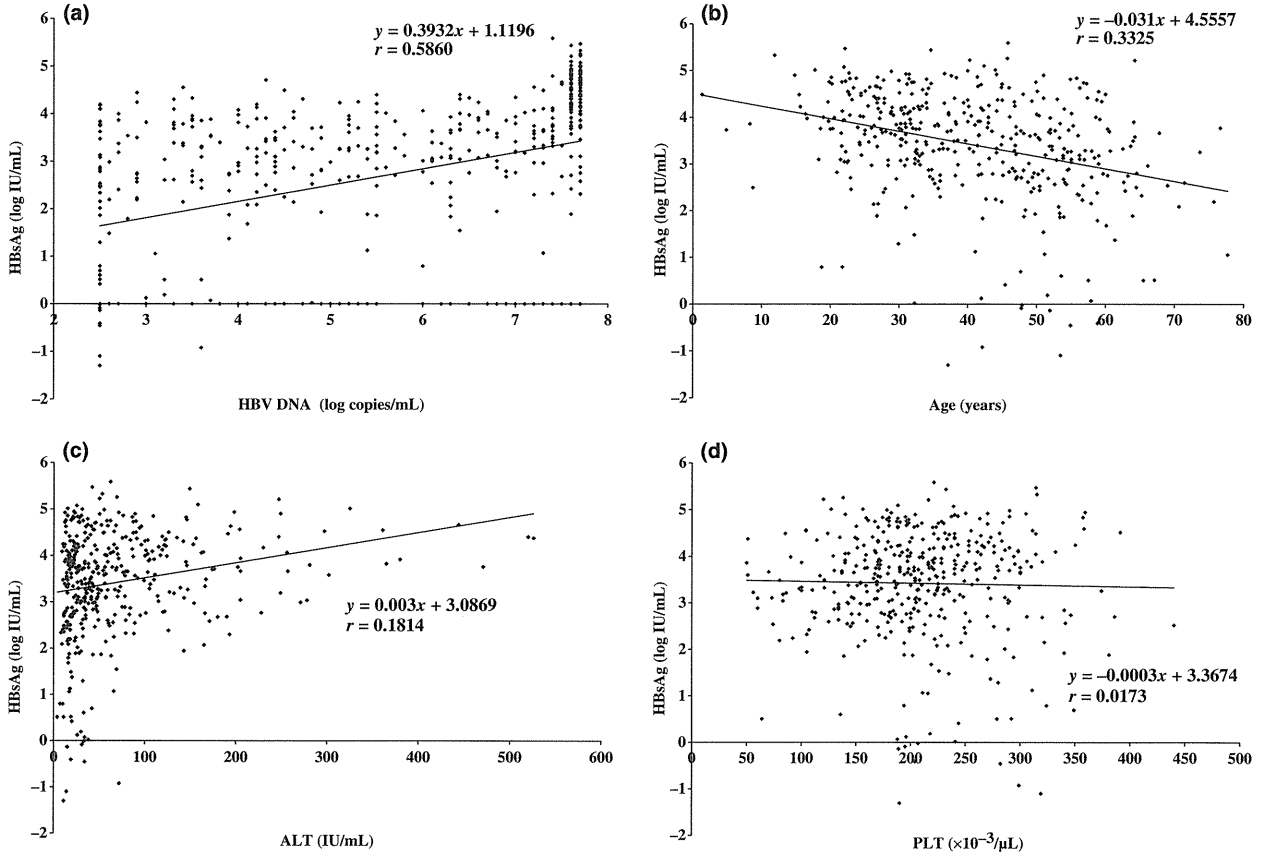

Fig. 2 Correlation between serum HBsAg levels and other clinical markers. (a) HBV DNA levels (Pearson's product moment correlation coefficient analysis; $r = 0.586$, $P < 0.001$), (b) age ($r = 0.333$, $P < 0.001$), (c) serum ALT levels ($r = 0.181$, $P < 0.001$), (d) the number of platelets ($r = 0.017$, $P = 0.347$). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

evaluated every year. The annualized rate of occurrence of HCC was 0.41% and one patient died of HCC and liver failure, although the death caused from liver failure without HCC was not observed. Seroconversion of HBeAg occurred during the follow-up of 61 patients (average age 32.8 ± 12.5 years). Antiviral drugs were used in 34 patients during follow-up. Of the 120 HBe-positive patients, 34 did not show HBeAg seroconversion and were not given antiviral drugs. Although HBsAg in these patients tended to decrease gradually year-by-year, there was a significant difference only after 5, 9 and 10 years from entry (Mann–Whitney U test, $P < 0.05$) (Fig. 3a). After the start of antiviral drugs, the level of HBsAg showed a significant decrease with statistical difference (paired t-test, $P = 0.035$) (Fig. 3b). Interestingly, in the patients in whom HBeAg seroconversion occurred during the natural course, the changes in HBsAg levels per year showed a significant decrease 2 years before seroconversion compared with the previous year (paired t-test, $P < 0.05$) (Fig. 3c). In addition, the levels of HBsAg showed a significant decrease after HBeAg seroconversion (paired t-test, $P = 0.035$).

The serial change in HBsAg levels before and after HBeAg seroconversion compared with the age- and sex-matched controls

Seroconversion of HBeAg has been reported to be influenced by gender [13], and in addition, from our analysis, the levels of HBsAg showed a gradual decrease. Therefore, we performed a control study, matched with age and sex, between two groups with and without HBeAg seroconversion during follow-up period. We extracted the patients who were matched for age and sex and compared 18 who did not show seroconversion through the course to 21 who showed seroconversion spontaneously, without treatment with a nucleotide analogue or interferon (IFN). A significant difference was not found in clinical background in this control study (Table 2). The changes in HBsAg levels in the groups with and without HBeAg seroconversion are shown in Fig. 4a. The level of HBsAg in the two groups gradually decreased over time, but the decline of HBsAg in the patients without HBeAg seroconversion was not significant over the course of a year. On the contrary, in the patients in whom

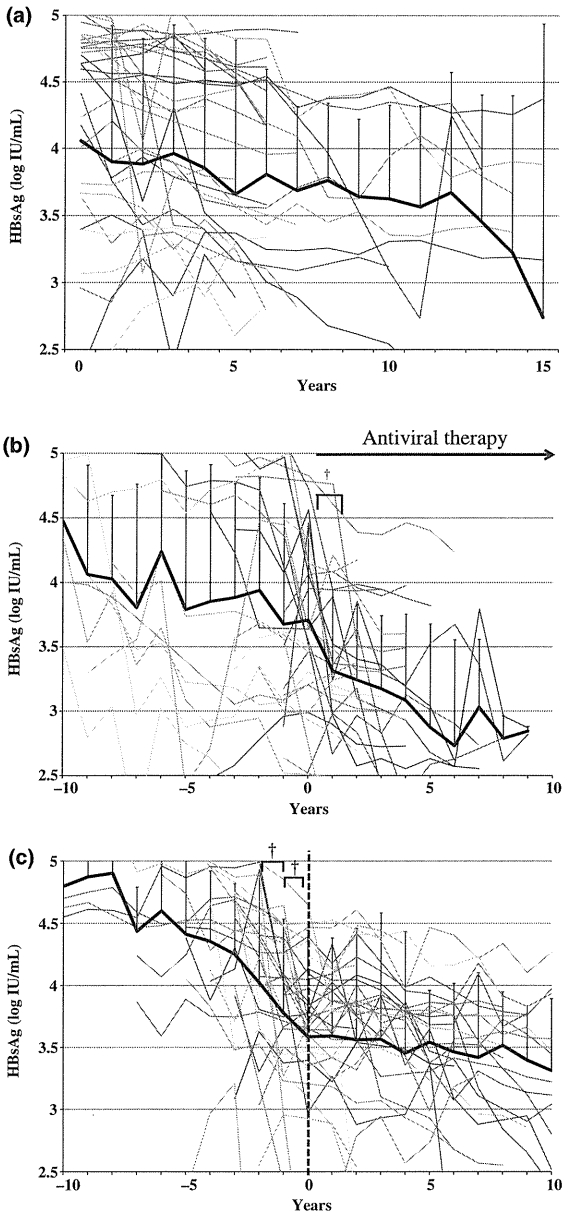

Fig. 3 The serial change of HBsAg level in HBeAg-positive patients with (a) no HBeAg seroconversion and no use of antiviral drugs (n = 34), (b) the use of antiviral drugs (n = 32), (c) HBeAg seroconversion during the follow-up period (n = 35). (a) Compared with the level at entry, a continuous decrease was not observed, although there was a significant difference only after 5, 9 and 10 years from entry ($P < 0.05$, Mann–Whitney U test). (b) The level of HBsAg showed a statistically significant decrease after commencement of antiviral therapy (†$P < 0.05$, paired t-test). (c) HBsAg showed a significant decrease at 2 years before seroconversion (†$P < 0.05$, paired t-test). HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

HBeAg seroconversion occurred during the natural course, the changes in HBsAg levels per year showed a significant decrease 2 years before seroconversion compared with the previous year (paired t-test, $P < 0.05$) (Fig. 4a). Next, we compared the difference in HBsAg levels between the two groups. There was a significant difference between the two groups 1 year before, on and after HBeAg seroconversion (unpaired t-test, $P < 0.05$). The HBeAg titre did not differ significantly between the two groups before seroconversion (Fig. 4b). The levels of HBcrAg showed an obvious decrease after HBeAg seroconversion, but before this, there was no significant decrease in the patients with or without HBeAg seroconversion (Fig. 4c).

DISCUSSION

The natural history of CHB is typically regarded as consisting of some phases that have been classified mainly by serum ALT levels, HBeAg and HBsAg serostatus, and HBV DNA levels. The understanding of the natural history of CHB has been facilitated by the improved sensitivity of serological and virological makers. HBsAg was the first HBV-encoded protein to be discovered [14]. Detection of HBsAg in serum is the fundamental diagnostic marker of HBV infection. HBsAg is a component of the Dane particle, which contains the viral genome, and subviral particles, but the mechanisms that regulate the production of HBsAg, particularly the subviral particles, are largely unclear [15]. Excess HBsAg may serve as a possible mechanism for evading the host immune responses, in that anti-HBs antibodies provide protective immunity [16]. One of our aims was to determine the change of HBsAg levels during the natural history of infection. Thompson *et al.* [17] reported that the level of HBsAg was related to the HBeAg status, as seen here. Some studies reported that positive correlations have been observed between the level of HBsAg and serum HBV DNA [18,19], again as seen here, but another study reported no such correlation [20]. Regarding the relationship with age, Kohmoto *et al.* [19] reported that the level of HBsAg was negatively correlated with the patient's age. We also found a weak and negative correlation between the levels of HBsAg and age, but in the analysis only of HBeAg-positive patients who did not show HBeAg seroconversion and who were not treated with antiviral drugs during follow-up period, the serial change of HBsAg levels showed no obvious decrease. Thus, the patients' age might have a direct effect on the level of HBsAg, but clinical events such as HBeAg seroconversion or the treatment of antiviral drugs might have a greater impact. Some studies reported that the level of HBsAg showed the difference among HBV genotypes [20,21]. In fact, we showed that the level of HBsAg in the patients with HBV genotype B was less than genotype C, but a limitation of this study was that most HBV carriers in our analysis were infected with genotype C of HBV. Therefore, we could not clarify the difference of HBsAg level among genotypes during HBeAg seroconversion.

In this study, a high HBsAg level was not related to the high incidence of HCC. In contrast, age, PLTs and the HBV