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Research Article

Long-Term Follow-Up of Hepatitis B e Antigen-Negative Hepatitis B Virus Carriers according to the Status of HBV DNA and ALT Levels -

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STRUCTURED ABSTRACT

Background and Aims: We examined the long-term prognosis of hepatitis B e antigen (HBeAg)-negative carriers according to the status of HBV DNA and ALT levels.

Methods: A total of 198 HBeAg-negative carriers visiting Chiba University Hospital between 2002 and 2005 were followed retrospectively for a median duration of 7.6 years. They were divided into 4 groups according to their maximal levels of HBV DNA and ALT, and were evaluated three times or more during the first year of follow-up: group A: 68 patients with HBV DNA <4 log copies (LC)/ml and ALT ≤30IU/l, group B: 31 with HBV DNA <4LC/ml and ALT >30IU/l, group C: 35 with HBV DNA ≥4LC/ml and ALT ≤30IU/l, group D: 64 with HBV DNA ≥4LC/ml and ALT >30IU/l.

Results: Hepatocellular Carcinoma (HCC) developed in 1, 0, 2 and 6 patients in groups A, B, C and D, respectively. These 9 patients were all cirrhotic except for 1 each in groups C and D. A significant factor associated with HCC development was cirrhosis, hepatitis flare-up, HBcrAg, AST, G-GTP, AFP, HBV DNA levels and platelet counts. Two patients died during follow-up, 1 of gastrointestinal bleeding and the other of HCC. Among 68 patients in group A, none developed hepatitis flare-up defined as ALT >80 IU/L and HBV DNA ≥4LC/ml.

Conclusions: HBeAg-negative carriers with HBV DNA <4 LC/ml and ALT ≤30 during the first year of follow-up showed favorable prognosis if inactive cirrhosis could be excluded.

Keywords: Hepatitis B virus; Hepatitis B e antigen-negative carrier; Hepatocellular carcinoma; Inactive carrier; Prognosis.

ABBREVIATIONS

HBeAg: Hepatitis B e Antigen; HBV: Hepatitis B Virus; HBV DNA: Hepatitis B Virus Deoxyribonucleic acid; HBcrAg: Hepatitis B core-related Antigen; HBsAg: Hepatitis B Surface Antigen; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; G-GTP: γ-Glutamyl Transpeptidase; APRI: Aspartate Aminotransferase to Platelet Ratio Index; AFP: α-Fetoprotein; BMI: Body Mass Index; CLEIA: Chemiluminescence Enzyme Immunoassay; ELISA: Enzyme-Linked Immunosorbent Assay; ROC: Receiver Operating Characteristic.

INTRODUCTION

Hepatitis B Virus (HBV) infection is a world-wide health problem, especially in Asia, with persistent infection of an estimated 240 million people, causing a variety of liver diseases including acute hepatitis, fulminant hepatitis, chronic hepatitis, cirrhosis and hepatocellular carcinoma [1].

The natural history of HBV infection has 5 major phases: immune tolerant, immune clearance; HBeAg-positive active infection, low replicative; HBV inactive carrier, reactivation; HBeAg-negative active infection, and HBsAg loss/occult infection [2]. Because HBsAg loss, one of the most important end-points in the antiviral treatment of chronic hepatitis B, is a rare event, around one percent /year [3-6], the low replicative phase is considered to be a common end-point of chronic hepatitis B in practical settings. Low replicative phase, known as inactive carrier with anti-HBe, was defined as being anti-HBe positive, with HBV DNA <4 log copies/ml, persistently normal levels of ALT, and absence of significant hepatitis by liver biopsy [7,8]. However, patients with probable inactive carrier sometimes show fluctuation of HBV DNA and ALT levels during follow-up, and liver biopsy is sometimes difficult to perform during routine examinations, which makes the diagnosis of inactive carrier difficult in practical settings.

Previous reports showed that 10 to 30% of probable inactive carriers experienced one or more reactivations of anti-HBe-positive hepatitis, characterized by a rise in HBV DNA > 4 log copies/ml accompanied by ALT elevations [9-12].

We investigated the prognosis of HBeAg-negative carriers for a retrospective median of 7.6 years after dividing them into 4 groups

according to the status of HBV DNA and ALT levels during the first year of follow-up in the present study.

METHODS

Patients

Patients of 454 consecutive HBsAg-positive patients who visited Chiba University Hospital between January 2002 and December 2005, a total of 198 patients were enrolled in this study. The inclusion criteria were HBsAg positive and HBeAg negative for more than 6 months with no restrictions on HBV DNA levels. The criteria for exclusion were: 1) follow-up period less than 13 months, 2) co-infection with hepatitis C virus, hepatitis D virus or human immunodeficiency virus, 3) autoimmune hepatitis, primary biliary cholangitis, hemochromatosis, Wilson's disease, or alcoholic liver disease, 4) hepatocellular carcinoma, 5) previous treatment with antiviral drugs such as interferon or nucleos(t)ide analogues, 6) complications such as chronic renal failure, collagen diseases or extrahepatic malignancies.

The diagnosis of cirrhosis was based on histological findings by liver biopsy or abdominal ultrasonographical findings, together with clinical features such as the presence of oesophageal varices, or platelet counts less than 10⁵/μl because of hypersplenism [13].

They were followed for a median of 7.6 years (range, 1.2-9.1 years). The follow-up was continued until the last visit, administration of antiviral drugs, HCC development, or the patient's death.

The definition of an inactive carrier was proposed by the Japan Society of Hepatology as follows: remaining HBeAg negative, serum ALT ≤30 IU/l and HBV DNA <4 log copies/ml, evaluated at least three times during follow-up of at least one year [14]. Therefore, patients were classified into 4 groups according to the maximal levels of HBV DNA and ALT during the 1st year of follow-up, with evaluation 3 times or more during the first year: group A, 68 patients with HBV DNA <4 log copies/ml and ALT ≤30 IU/l; group B, 31 with HBV DNA <4 log copies/ml and ALT >30 IU/l; group C, 35 with HBV DNA ≥4 log copies/ml and ALT ≤30 IU/l; group D, 64 with HBV DNA ≥4 log copies/ml and ALT >30 IU/l (Table 1). The frequency of total evaluations during follow-up was 1.92/year, 2.45/year, 2.69/year and 3.81/year in groups A, B, C, and D, respectively. The frequency of evaluations after the first year of follow-up was 1.72/year, 2.30/year, 2.50/year and 3.50/year in groups A, B, C and D, respectively.

This study was approved by the ethics committee of Chiba University, Japan (permission number 1375), and conformed to the Helsinki Declaration.

Laboratory data

HBV DNA was quantified by Amplicore monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6 to 7.6 log copies/ml. The HBsAg level was measured using the Chemiluminescence Enzyme Immunoassay (CLEIA)-based HISCLR HBsAg assay (Sysmex Co. Ltd., Kobe, Japan) with a range of -1.5 to 3.3 log IU/ml. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. The HBcrAg level was measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fuji Rebio Inc., Tokyo, Japan) with a range of 3.0 to 6.8 log U/ml. The HBeAg and anti-HBe levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA; Abbott Laboratory, Chicago, IL, USA). The six major genotypes of HBV (A–F) were determined by ELISA (HBV Genotype EIA; Institute of Immunology Co. Ltd., Tokyo, Japan). APRI (AST to platelet ratio index) was calculated as AST level (IU/l)/upper limit of normal of AST level (IU/l)/platelet count (10⁹/L) x100 [15], and the FIB-4 index was calculated as [age (years) x AST level (IU/l)] / [platelet count (10⁹/L) x ALT level (IU/L)] [16].

Imaging methods to detect HCC

Abdominal ultrasonography was performed at least every 6 to 12 months to detect HCC, and if the possibility of HCC existed,

further evaluation, such as by computed tomography, magnetic resonance imaging, hepatic angiography, or tumor biopsy guided by ultrasonography, was performed.

STATISTICAL ANALYSIS

Mann Whitney U-test or Student’s t test and Fisher’s exact test were used to analyze quantitative and qualitative data, respectively. Cumulative occurrence curves of HCC development and hepatitis flare-up were determined by the Kaplan-Meier method and the differences between groups were assessed by the log-rank test. The diagnostic performance of HBsAg titers and HBV DNA levels was evaluated by Receiver Operating Characteristic (ROC) curve. Statistical procedures were performed using Stat View statistical software version 5 (SAS Institute Inc.). P values <0.05 were considered significant.

RESULTS

Comparison of baseline characteristics among groups A, B, C and D

Among 99 patients with HBV DNA <4 log copies/ml (groups A and B), 31 patients with ALT >30 IU/l (group B) showed higher frequency of male gender, higher levels of BMI and AFP, and also higher AST, ALT, G-GTP levels and APRI scores at enrolment, but there was no difference in HBV DNA levels between the two groups (Table 1). Furthermore, among 64 patients in group A and 29 in group B, in whom abdominal ultrasonography was conducted at enrolment, patients in group B showed higher frequency of fatty liver than those in group A (11/64, 17.2% vs 13/29, 44.8%, p = 0.0093).

Table 1: Baseline characteristics of anti-HBe antibody-positive patients according to the levels of HBV DNA and ALT during the 1st year of follow-up.

	Group A (HBV DNA<4 & ALT ≤ 30)	Group B (HBV DNA<4 & ALT>30)	Group C (HBV DNA≥4 & ALT ≤ 30)	Group D (HBV DNA≥4 & ALT>30)	Groups A vs B	Groups C vs D	Groups A vs C	Groups A vs D	Groups B vs C	Groups B vs D
Patient number, n	68	31	35	64						
Age (range), years	45 (5, 73)	46 (7, 69)	50 (15, 86)	47 (23, 72)	0.6371	0.4595	0.3916	0.7724	0.2499	0.4946
Male (%)	29 (43)	26 (84)	12 (34)	38 (59)	0.0002	0.0213	0.5245	0.5850	<0.0001	0.0200
BMI (range), kg/m ²	21.3 (16.7, 30.3)	24.3 (16.9, 31.2)	21.5 (18.1, 34.7)	22.9 (16.7, 29.7)	0.0207	0.1233	0.7214	0.2158	0.0145	0.0719
Genotype (A/B/C/D/ UD)	3/10/44/1/8	1/1/23/0/5	1/10/24/0/0	2/15/43/1/2	0.4552	0.7588	0.1343	0.3146	0.0071	0.0321
HBV DNA (range), log copies/ml	2.8 (<2.6, 3.9)	<2.6 (<2.6, 3.5)	4.1 (<2.6, 5.9)	5.2 (3.3, >7.6)	0.0929	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HBcrAg (range), logU/ml	<3.0 (<3.0, 5.5)	<3.0 (<3.0, >6.8)	<3.0 (<3.0, 6.0)	<3.0 (<3.0, >6.8)	0.2129	<0.0040	0.9511	<0.0001	0.9511	0.0070
HBsAg (range), logIU/ml	3.2 (-1.2, 4.6)	2.6 (-1.2, 4.6)	3.4 (1.9, 4.6)	3.4 (-0.5, 4.9)	0.5736	0.4124	0.1302	0.0446	0.1302	0.0167
AST (range), IU/l	19 (11, 32)	26 (15, 84)	21 (10, 29)	33 (16, 133)	<0.0001	<0.0001	0.0043	<0.0001	0.0043	0.0471
ALT (range), IU/l	16 (7, 28)	32 (13, 143)	18 (6, 27)	41 (14, 153)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0747
G-GTP (range), IU/l	16 (6, 71)	32 (13, 124)	16 (8, 54)	29 (12, 242)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3446
AFP (range), ng/ml	2.6 (0.5, 10.4)	3.0 (1.3, 19.8)	2.6 (1.2, 23.6)	3.1 (1.2, 105)	0.0434	0.3212	0.2775	0.0097	0.4894	0.8423
Platelets (range), x10 ⁴ /μl	21.7 (7.2, 40.0)	23.2 (6.5, 40.7)	23.1 (9.6, 33.4)	20.2 (5.1, 34.0)	0.4688	0.0191	0.6470	0.0168	0.8903	0.0137
APRI	0.279(0.108, 0.715)	0.361 (0.112, 1.248)	0.287(0.101, 0.884)	0.485(0.166, 4.040)	0.0005	<0.0001	0.5969	<0.0001	0.0051	0.0087
FIB-4 index	1.020 (0.060, 4.130)	0.960 (0.140, 3.020)	1.035 (0.400, 3.590)	1.240(0.360, 8.650)	0.8474	0.2796	0.6598	0.0524	0.4822	0.0789
Cirrhosis (%)	2 (2.9)	4 (12.9)	1 (2.9)	7 (10.9)	0.0749	0.2536	>0.9999	0.0893	0.1786	0.7452
Follow-up period (month)	97 (15, 109)	96 (14, 108)	89 (36, 109)	80 (16, 109)	0.1044	0.1179	0.1602	0.0015	0.6026	0.3590
NA treatment	0	0	7	17	-	0.6246	0.0003	<0.0001	0.0120	0.0010

HBV, hepatitis B virus; BMI, body mass index; UD, undetermined; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; G-GTP, γ-glutamyl transpeptidase; APRI, aspartate aminotransferase to platelet ratio index; AFP, α-fetoprotein, NA, nucleos(t)ide analogues. The number of patients who received NA treatment during the follow-up period are shown.



Among 99 patients with HBV DNA ≥ 4 log copies/ml (groups C and D), 64 patients with ALT >30 IU/l (group D) showed higher frequency of male gender, higher levels of HBV DNA and HBcrAg, lower platelet count, in addition to higher levels of AST, ALT, G-GTP and APRI scores than 35 patients with ALT ≤ 30 IU/l (group C) at enrolment (Table 1).

Among 103 patients with ALT ≤ 30 IU/l (groups A and C), there were no difference between the 2 groups except for HBV DNA levels and NA treatment (Table 1). Among 95 patients with ALT >30 IU/l (groups B and D), 64 patients with HBV DNA ≥ 4 log copies/ml (group D) showed higher levels of HBV DNA, HBcrAg, HBsAg and APRI (Table 1).

Median follow-up period was 97, 96, 89 and 80 months in groups A, B, C and D, respectively. The number of patients who received nucleos(t)ide analogues treatment during the follow-up period was 0, 0, 7 and 17 in groups A, B, C and D, respectively (Table 1) or 20 of 184 non-cirrhotic and 4 of 14 cirrhotic patients. There were no patients who received interferon during the follow-up period.

Hepatitis flare-up

Hepatitis flare-up defined as ALT >80 IU/l and HBV DNA ≥ 4.0 log copies/ml was experienced by 0, 0, 4 and 31 patients in groups A, B, C and D, respectively. Cumulative occurrence curves of hepatitis flare-up were determined in groups A, B, C and D by the Kaplan-Meier method (Figure 1). The cumulative incidence of hepatitis flare-up in 1, 3 and 5 years was 2.9%, 8.6% and 11.8% in group C, and 32.8%, 44.3% and 48.3% in group D, respectively. Hepatitis flare-up was observed in 10 patients (15.6%) of group D at enrolment.

Distributions of maximal and minimal levels of HBV DNA in groups A, B, C and D from 1 year after enrolment to last follow-up

Maximal and minimal levels of HBV DNA in each patient from 1 year after enrolment to last follow-up were evaluated in patients of groups A, B, C and D (Table 2). Fifty-one patients (75%) in groups A and 27 patients (87%) in group B showed persistent HBV DNA <4.0 log copies/ml and all patients in groups A and B showed persistent HBV DNA <5 log copies/ml except 1 patient in group A. On the other hand, 6 patients (17%) in group C and 1 patient (2%) in group D showed persistent HBV DNA <4.0 log copies/ml and 4 (12%) in group C and 24 (37%) in group D showed persistent HBV DNA ≥ 4 log copies/ml, and 31 (89%) in group C and 40 (63%) in group D showed transient HBV DNA <4.0 log copies/ml (Table 2).

Change of HBV DNA and ALT levels during follow-up in patients with HBV DNA <4 log copies/ml and ALT ≤ 30 IU/l at enrolment

The number of patients with HBV DNA <4 log copies/ml and ALT levels ≤ 30 IU/l at enrolment was 88, excluding 5 cirrhotic patients. Sixty-six (75%), 9 (10%) and 13 (15%) of these patients belonged to groups A, B and C, respectively, with none in group D. Thirty-four of 66 (51.5%) patients in group A showed HBV DNA <4 log copies/ml and ALT ≤ 30 IU/l throughout the follow-up period. Therefore, only 34 of 88 (38.6%) patients persistently showed HBV DNA <4 log copies/ml and ALT ≤ 30 IU/l during follow-up. In group A, 4 patients showed elevations of both HBV DNA ≥ 4 log copies/ml and ALT >30 IU/l during follow-up, but hepatitis flare-up defined as ALT >80 IU/l and HBV DNA ≥ 4 log copies/ml was not detected in these patients. On the other hand, among 88 with HBV DNA <4 log copies/ml and

ALT ≤ 30 IU/l at enrolment, 11 patients showed elevations of both HBV DNA ≥ 4 log copies/ml and ALT >30 IU/l, and 2 of them showed hepatitis flare-up during follow-up.

Prediction of patients with persistent HBV DNA <4 log copies/ml

In 88 non-cirrhotic patients with HBV DNA <4 log copies/ml and ALT ≤ 30 IU/l at enrolment, baseline characteristics were compared between 58 patients with persistent HBV DNA <4 log copies/ml and 30 with transient HBV DNA ≥ 4 log copies/ml during follow-up. The former group (58 patients) showed significantly lower levels of HBV DNA and more frequent male gender compared to the latter group (Table 3). HBsAg titers in the former group were lower than in the latter group with marginal significance ($p = 0.0632$). The cut-

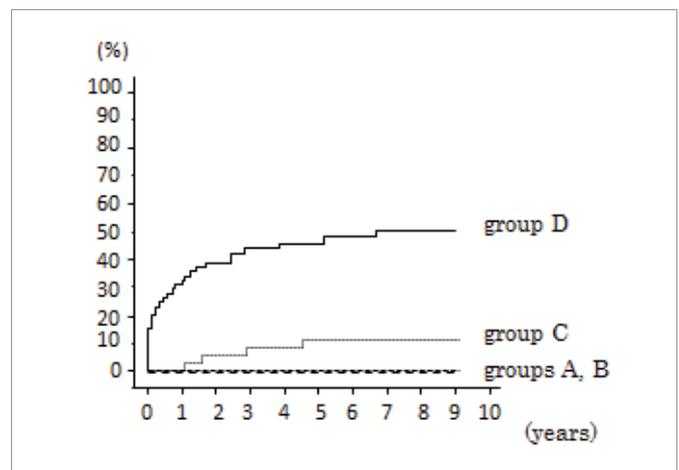


Figure 1: Cumulative occurrence curves of hepatitis flare-up in groups A, B, C and D. P value was 0.0002 by log-rank test between groups C (n=35) and D (n=64).

Table 2: Maximal and minimal levels of HBV DNA in groups A, B, C and D from 1 year after enrolment to last follow-up.

	Group A (N = 68)	Group B (N = 31)	Group C (N = 35)	Group D (N = 64)
Maximal HBV DNA (log copies/ml)				
<4.0	51 (75%)	27 (87%)	6 (17%)	1 (2%)
$\geq 4.0, <5.0$	16 (24%)	4 (13%)	18 (51%)	15 (23%)
$\geq 5.0, <6.0$	1 (1%)	0	5 (14%)	15 (23%)
$\geq 6.0, <7.0$	0	0	4 (11%)	14 (22%)
≥ 7.0	0	0	2 (6%)	19 (30%)
Minimal HBV DNA (log copies/ml)				
<2.6	52 (76%)	27 (87%)	16 (46%)	10 (16%)
$\geq 2.6, <4.0$	16 (24%)	4 (13%)	15 (43%)	30 (47%)
$\geq 4.0, <5.0$	0	0	3 (9%)	14 (22%)
$\geq 5.0, <6.0$	0	0	1 (3%)	6 (9%)
≥ 6.0	0	0	0	4 (6%)

Group A: 68 patients with HBV DNA <4 log copies/ml and ALT ≤ 30 IU/l during the first year of follow-up.
Group B: 31 patients with HBV DNA <4 log copies/ml and ALT >30 IU/l during the first year of follow-up.
Group C: 35 patients with HBV DNA ≥ 4 log copies/ml and ALT ≤ 30 IU/l during the first year of follow-up.
Group D: 64 patients with HBV DNA ≥ 4 log copies/ml and ALT >30 IU/l during the first year of follow-up.



off value of HBV DNA level and HBsAg titer for HBV DNA level elevation equal to or greater than 4 log copies/ml was 3.0 log copies/ml (AUROC=0.6805, 95% CI: 0.5608-0.8002, $p = 0.0031$) and 3.05 log IU/ml (AUROC = 0.6216, 95% CI: 0.5059-0.7374, $p = 0.0395$) by ROC curve analysis, respectively. HBsAg levels were below 3.05 log IU/ml in 31 patients (53.4%) of the former group and in 8 patients (26.7%) of the latter group ($p = 0.0233$). The diagnostic performance of the 3.05 log IU/ml cut-off to identify an inactive carrier showed sensitivity of 53%, specificity of 73%, and diagnostic accuracy of 60%, while that of the 2.0 log IU/ml cut-off showed sensitivity of 31%, specificity of 97%, and diagnostic accuracy of 53%.

Development of hepatocellular carcinoma

Hepatocellular Carcinoma (HCC) developed in 9 patients: 1 in group A, 2 in group C, 6 in group D. They were all cirrhotic except for 2 patients, 1 in group C and 1 in group D. These 9 patients with HCC showed significantly higher levels of HBV DNA, HBcrAg, AST, G-GTP, AFP, and lower platelet counts and higher frequency of cirrhosis at enrolment and higher frequency of hepatitis flare-up during follow-up than the 189 patients without development of HCC (Table 4). The cumulative occurrence curves of HCC development were determined according to cirrhotic status by the Kaplan-Meier method (Figure 2). There were no patients who developed HCC after HBsAg loss during the follow-up period in this study.

Cause of death

Overall death occurred in 2 patients, one cirrhotic in group B and one non-cirrhotic in group D. The cause of death was gastrointestinal bleeding and HCC, respectively.

Table 3: Baseline characteristics of patients with maximal HBV DNA <4 log copies/ml and with maximal HBV DNA ≥4 log copies/ml during follow-up among non-cirrhotic patients with HBV DNA <4 log copies/ml and ALT ≤30 IU/l at enrolment.

	Maximal HBV DNA<4 log copies/ml	Maximal HBV DNA≥4 log copies/ml	p-value
Patient number, n	58	30	
Age (range), years	46 (5, 73)	45 (22, 86)	0.8086
Male, n (%)	29 (50)	7 (23)	0.0219
BMI (range), kg/m ²	22.3 (16.7, 30.3)	20.7 (17.7, 31.2)	0.0755
Genotype (A/B/C/D/UD)	2/8/35/1/10	3/6/20/0/0	0.0998
HBV DNA (range), log copies/ml	2.7 (<2.6, 3.9)	3.1 (<2.6, 3.9)	0.0047
HBcrAg (range), logU/ml	<3.0 (<3.0, 5.5)	<3.0 (<3.0, 6.0)	0.5757
HBsAg (range), logIU/ml	3.0 (-1.2, 4.6)	3.5 (2.0, 4.4)	0.0632
AST (range), IU/l	20 (11, 34)	22 (10, 29)	0.5570
ALT (range), IU/l	17 (7, 30)	17 (6, 27)	0.5516
G-GTP (IU/l)	16 (6, 71)	16 (8, 54)	0.3633
Platelets (range), x10 ⁴ /μl	22.8 (14.7, 40.7)	23.0 (16.5, 33.4)	0.4676
APRI	0.285 (0.108, 0.524)	0.247 (0.101, 0.433)	0.7647
FIB-4 index	0.990 (0.060, 2.600)	0.950 (0.350, 2.810)	0.9579
AFP (range), ng/ml	2.6 (0.5, 10.4)	3.1 (1.2, 23.6)	0.3139

HBV: Hepatitis B Virus; BMI: Body Mass Index; UD: Undetermined; HBcrAg: Hepatitis B Core-related Antigen; HBsAg: Hepatitis B Surface Antigen; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; G-GTP: γ-Glutamyl Transpeptidase; APRI: Aspartate Aminotransferase to Platelet Ratio Index; AFP: α-Fetoprotein.

Table 4: Baseline characteristics between patients with and without HCC development.

	With HCC development	Without HCC development	p-value
Patient number	9	189	
Age (range), years	54 (41, 64)	46 (5, 86)	0.0948
Male, n (%)	5 (56)	100 (53)	>0.9999
BMI (range), kg/m ²	22.4 (18.2, 28.7)	22.5 (16.7, 34.7)	0.9508
Genotype (A/B/C/D/UD)	0/0/9/0/0	7/36/125/2/15	0.3763
HBV DNA (range), log copies/ml	4.6 (3.5, 6.6)	3.4 (<2.6, >7.6)	0.0059
HBcrAg (range), logU/ml	5.1 (<3.0, 5.8)	<3.0 (<3.0, >6.8)	0.0088
HBsAg (range), logIU/ml	3.9 (0.7, 4.0)	3.2 (-1.2, 4.9)	0.2384
AST (range), IU/l	42 (17, 68)	23 (10, 133)	0.0232
ALT (range), IU/l	45 (11, 57)	22 (6, 153)	0.1001
G-GTP (range), IU/l	51 (12, 242)	20 (6, 144)	0.0209
AFP (range), ng/ml	6.7 (1.9, 104.9)	2.7 (0.5, 19.8)	0.0149
Platelets (range), x10 ⁴ /μl	11 (5.1, 20.1)	22.2 (6.5, 40.7)	<0.0001
Cirrhosis, n (%)	7 (78)	7 (3.7)	<0.0001
Hepatitis flare-up, n (%)	5 (56)	30 (16)	0.0097

HCC: Hepatocellular Carcinoma; BMI: Body Mass Index; UD: Undetermined; HBV: Hepatitis B Virus; HBcrAg: Hepatitis B Core-related Antigen; HBsAg: Hepatitis B Surface Antigen; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; G-GTP, γ-Glutamyl Transpeptidase; AFP: α-Fetoprotein
Hepatitis flare-up defined as ALT >80 IU/l and HBV DNA ≥4 log copies/ml.

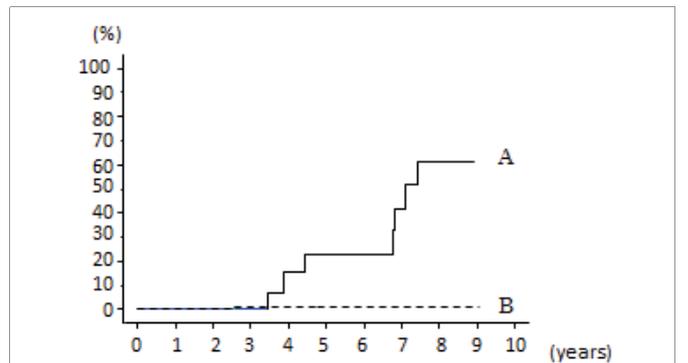


Figure 2: Cumulative occurrence curves of HCC development in cirrhotic (A) and non-cirrhotic patients (B). P value was <0.0001 by log-rank test between cirrhotic (n=14) and non-cirrhotic (n=184) patients.

DISCUSSION

We investigated HCC development and survival, and changes of HBV DNA and ALT levels in HBsAg-negative carriers with a median follow-up of 7.6 years, and we found that HBV carriers in group A (HBV DNA <4 log copies/ml and ALT ≤30 IU/l during the first year of follow-up) showed no death, no development of HCC and no hepatitis flare-up if inactive cirrhosis was excluded, although half of these patients showed transient elevation of HBV DNA levels equal to or greater than 4 log copies/ml.

ALT elevation is usually accompanied by HBV DNA above 5 to 6 log copies/ml in chronic hepatitis B. In the present study, patients in group B had higher frequency of fatty liver and higher levels of BMI than those in group A. Therefore ALT elevation more than 30 IU/l in group B would not be due to hepatitis B but rather to other causes

such as fatty liver, as shown in a population-based cohort study by Spradling et al. [17].

An inactive carrier state is defined as negative for HBeAg, HBV DNA <4 log copies/ml and persistent ALT lower than the upper limit of normal with minimal or no necroinflammation and minimal to mild fibrosis on liver biopsy [7,8]. As liver biopsy is not easy to perform in clinical practice, the diagnosis of inactive carrier mainly depends on the results of blood tests, such as HBeAg/anti-HBe status and the levels of HBV DNA and ALT. However, the HBV DNA and ALT levels fluctuate during the course of HBV infection. In the present study, 25 (26.9%) of 93 patients with HBV DNA <4 log copies/ml and ALT \leq 30 IU/l at enrolment showed elevations of HBV DNA \geq 4 log copies/ml and/or ALT >30 IU/l during the first year of follow-up. Two of the 25 patients showed hepatitis flare-up defined as ALT > 80 IU/l and HBV DNA \geq 4 log copies/ml and one non-cirrhotic patient among the 25 patients, who belonged to group C, developed HCC during the follow-up period, while none in group A showed hepatitis flare-up. Furthermore, 3 of the 25 patients were treated with nucleot(s)ide analogues during follow-up while none of the patients in group A were treated with the antiviral drugs. Thus, it is evident that a single-point evaluation of HBV DNA and ALT levels is insufficient to select inactive carriers, but a one-year monitoring of HBV DNA and ALT levels consisting of three separate examinations would be reasonable for selecting inactive carriers.

Of course, accurate prediction of inactive carriers by a single-point evaluation is desirable, and recent reports showed serum HBsAg titers as a promising candidate [18-21]. In the present study, HBsAg titer at enrolment was significantly lower in group A than in group D ($p = 0.0446$), but among 88 non-cirrhotic patients with HBV DNA <4 log copies/ml and ALT \leq 30 IU/l at enrolment, HBsAg titer was marginally different between patients with persistent HBV DNA <4 log copies/ml and those with transient HBV DNA \geq 4 log copies/ml throughout the follow-up period (Table 3). A 2 log IU/ml cut-off of serum HBsAg identified inactive carriers with 97% specificity in the present study, but the diagnostic performance of the 3.05 log IU/ml cut-off to identify inactive carriers showed only 60% diagnostic accuracy, a much lower frequency compared to previous reports [18-21]. But this is comparable to the report by Park et al., in which genotype C HBeAg negative carriers were investigated and a HBsAg cut-off level of less than 850 IU/ml predicted inactive carriers with 63.5% diagnostic accuracy [22]. Our study showed that 27 patients with baseline HBsAg \geq 3.05 log IU/ml were significantly younger than 31 with HBsAg <3.05 log IU/ml (37.4 ± 15.8 vs 50.8 ± 15.1 , $p = 0.0018$) among the 58 patients with persistent HBV DNA <4 log copies/ml. Therefore, evaluation of serum HBsAg titer might not be useful for the identification of younger HBeAg-negative inactive carriers.

HCC developed in 9 patients, and all but 2 were cirrhotic. Careful follow-up is necessary for cirrhotic patients with HBV DNA <4 log copies/ml and ALT \leq 30 IU/l, as one cirrhotic patient in group A developed HCC.

There were some limitations to this study. This is a retrospective study with a relatively small cohort size. Therefore, some selection bias might have affected the results. Still, the patients were consecutively enrolled if they had no exclusion criteria and blood examinations were performed regularly at one institution. Liver biopsy was not conducted in patients who were classified as inactive carriers because they were asymptomatic and had no clinical complications, and histologically significant liver disease was considered rare [23]. There were 24 patients who received NA treatment during the follow-up

period, 0, 0, 7 and 17 patients in groups A, B, C and D, respectively. Therefore this could be bias for evaluations of natural history in patients of groups C and D, but not of groups A and B. In the present study, the sample size of the HCC group was too small to apply Cox proportional hazards model, therefore we could not determine the most significant factors associated with HCC development.

In conclusion, favorable prognosis of inactive carriers was clearly shown in comparison to patients with HBeAg-negative chronic hepatitis, and careful follow-up of inactive cirrhosis with HBV DNA <4 log copies/ml and ALT \leq 30 is essential.

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CONFLICT OF INTEREST

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