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ORIGINAL ARTICLE

Observational Study

Occult hepatitis B virus infection is not associated with disease progression of chronic hepatitis C virus infection

Junhyeon Cho, Sang Soo Lee, Yun Suk Choi, Yejoo Jeon, Jung Wha Chung, Joo Yeong Baeg, Won Keun Si, Eun Sun Jang, Jin-Wook Kim, Sook-Hyang Jeong

Junhyeon Cho, Yun Suk Choi, Yejoo Jeon, Jung Wha Chung, Joo Yeong Baeg, Won Keun Si, Eun Sun Jang, Jin-Wook Kim, Sook-Hyang Jeong, Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Gyeonggi-do 463-707, South Korea

Sang Soo Lee, Department of Internal Medicine, Gyeongsang National University Changwon Hospital, Gyeongsang National University College of Medicine, Gyeonggi-do 463-707, South Korea

Author contributions: Cho JH, Lee SS and Jeong SH designed the study; Jang ES, Kim JW and Jeong SH enrolled the patients; Cho JH, Lee SS, Choi YS, Jeon Y, Kim JW, Baeg JY and Si WK collected the data; Cho JH, Lee SS and Jeong SH analyzed and interpreted the data; all authors read and approved the final manuscript; Cho JH and Lee SS contributed equally to this manuscript.

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Correspondence to: Sook-Hyang Jeong, MD, Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, 82 Gumiro 173, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, South Korea. jsh@snubh.org
Telephone: +82-31-7877029
Fax: +82-31-7874052

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Abstract

AIM

To clarify the prevalence of occult hepatitis B virus (HBV) infection (OBI) and the association between OBI and liver disease progression, defined as development of liver cirrhosis or hepatocellular carcinoma (HCC), worsening of Child-Pugh class, or mortality in cases of chronic hepatitis C virus (HCV) infection.

METHODS

This prospective cohort study enrolled 174 patients with chronic HCV infection (chronic hepatitis, n = 83; cirrhosis, n = 47; HCC, n = 44), and evaluated disease progression during a mean follow-up of 38.7 mo. OBI was defined as HBV DNA positivity in 2 or more

different viral genomic regions by nested polymerase chain reaction using 4 sets of primers in the S, C, P and X open reading frame of the HBV genome.

RESULTS

The overall OBI prevalence in chronic HCV patients at enrollment was 18.4%, with 16.9%, 25.5% and 13.6% in the chronic hepatitis C, liver cirrhosis and HCC groups, respectively (P = 0.845). During follow-up, 52 patients showed disease progression, which was independently associated with aspartate aminotransferase > 40 IU/L, Child-Pugh score and sustained virologic response (SVR), but not with OBI positivity. In 136 patients who were not in the SVR state during the study period, OBI positivity was associated with neither disease progression, nor HCC development.

CONCLUSION

The prevalence of OBI in chronic HCV patients was 18.4%, and OBI was not associated with disease progression in South Koreans.

Key words: Hepatitis B virus; Hepatitis C virus; Disease control; Oncogenesis

Core tip: Whether occult hepatitis B virus (HBV) infection affects the outcomes of chronic hepatitis C virus infection is controversial. This prospective observational study aimed to clarify the association between occult HBV infection and liver disease progression defined as development of liver cirrhosis, worsening of Child-Pugh class, hepatocellular carcinoma or mortality in patients with chronic hepatitis C infection in South Korea.

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INTRODUCTION

Prevalence of hepatitis C virus (HCV) infection involves about 3% of the global population, or approximately 170 million people[1]. Chronic HCV infection can lead to liver cirrhosis or hepatocellular carcinoma (HCC) in 20%-30% of cases[2-4]. The HCV life cycle occurs exclusively in the cell cytoplasm, and the HCV RNA genome does not integrate into the host genome[5,6]. Therefore, the pathogenic mechanisms of HCV include complex interaction of HCV proteins and host proteins, inducing chronic inflammation, inhibition of apoptosis, stimulation of cell proliferation and fibrosis, leading to genetic alteration of hepatocytes, liver cirrhosis and ultimately HCC[7]. In contrast, hepatitis B virus (HBV) has a direct oncogenic effect by integration of HBV DNA into the host genome, causing insertional mutagenesis, in addition to the indirect effects by HBV protein-host interaction.

HCV and HBV have similar transmission routes, and coinfection with HCV and HBV can increase the risk of HCC, compared to HCV monoinfection[8]. Even if serum hepatitis B surface antigen (HBsAg) is negative, HBV DNA can exist in the liver or blood of people in an occult HBV infection (OBI) state. The pathogenic role of OBI in the development of cirrhosis or HCC among patients with chronic HCV infection is still extremely controversial[9-12]. Moreover, the prevalence of OBI in chronic HCV-infected patients shows a wide range of variation in different global regions[13,14]. There are no data on the prevalence or effect of OBI in chronic HCV infection in South Korea, an HBV endemic region.

This study aimed to clarify the prevalence of OBI in the blood of patients with chronic HCV infection, and to estimate the association between OBI and liver disease progression defined as development of liver cirrhosis, decompensation (worsening of Child-Pugh class), HCC or mortality among the subjects by prospective observation. We also investigated the HBV genotype on the HBsAg-coding open reading frame of HBV gene (S-ORF) in OBI-positive patients.

MATERIALS AND METHODS

Subjects

This prospective cohort study included 174 consecutively enrolled patients with HBsAg-negative, chronic HCV infection in Seoul National University Bundang Hospital between November 2005 and May 2014. Chronic HCV infection was defined as HCV RNA positivity > 6 mo with HBsAg negativity. Among them, 83 patients were given diagnoses of chronic hepatitis C, 47 patients of liver cirrhosis, and 44 patients of HCC. The diagnostic criteria for liver cirrhosis included the presence of portal hypertension manifested as splenomegaly, thrombocytopenia < 100000/mm^3, ascites, varices, or hepatic encephalopathy and imaging findings compatible with liver cirrhosis. The diagnosis of HCC was based on histological examination or typical radiographic image findings consisting of arterial enhancement and venous wash-out of hepatic nodules on contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI)[15]. HBsAg-positive patients or human immunodeficiency virus coinfected patients were excluded. Informed consent was obtained from all patients, and the study protocol was approved by the Institutional Review Board of the hospital.

Table 1 Baseline characteristics of 174 patients with chronic hepatitis C virus infection

Variable	Total (*n* = 174)	Chronic hepatitis (*n* = 83)	Liver cirrhosis (*n* = 47)	HCC (*n* = 44)
Mean age, yr[b,c]	66.5 ± 9.9	65.6 ± 9.8	65.3 ± 10.1	69.7 ± 9.3
Male sex	105 (60.3)	54 (65.1)	24 (51.1)	27 (61.4)
Body mass index (kg/m^2)	23.3 ± 3.0	23.6 ± 3.0	23.0 ± 3.2	23.0 ± 2.8
Ex or current smoker (*n* = 173)	51 (29.5)	26 (31.3)	10 (21.7)	15 (34.1)
Alcohol intake (social or heavy) (*n* = 173)	80 (46.2)	42 (50.6)	17 (37.0)	21 (47.8)
Anti-HBc (*n* = 100)	75 (75.0)	35 (71.4)	18 (85.7)	22 (73.3)
Hemoglobin (g/dL)[c]	13.5 ± 1.8	14.1 ± 1.5	13.5 ± 1.9	12.4 ± 1.9
Platelet (× 103/μL)[a,b]	151.3 ± 87.4	188.0 ± 55.7	117.5 ± 120.2	118.3 ± 66.2
Albumin (g/dL)[a,b]	4.0 ± 0.5	4.2 ± 0.2	3.8 ± 0.5	3.7 ± 0.5
Total bilirubin (mg/dL)[a]	1.0 ± 0.6	1.0 ± 1.7	1.2 ± 0.6	1.1 ± 0.8
ALP (IU/L)[a,b]	100.3 ± 47.3	89.2 ± 30.9	102.0 ± 37.0	119.3 ± 71.4
AST (IU/L)[a,b]	75.8 ± 103.3	76.0 ± 141.9	72.6 ± 47.8	78.8 ± 45.3
ALT (IU/L)	77.2 ± 138.5	89.7 ± 193.2	68.0 ± 59.3	63.3 ± 41.0
Creatinine (mg/dL)[a]	0.9 ± 0.4	1.0 ± 0.5	0.8 ± 0.2	0.9 ± 0.2
Prothrombin time (INR)[a,b]	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
AFP > 20 ng/mL (*n* = 171)[a,b]	30 (17.5)	5 (6.2)	9 (19.6)	16 (36.4)
Child-Pugh class (A/B/C)[a,b]				
A	160 (92.0)	83 (100)	40 (85.1)	37 (84.1)
B	12 (6.9)	0	5 (10.6)	7 (15.9)
C	2 (1.1)	0	2 (4.3)	0
MELD score (*n* = 167)[a,b]	8.5 ± 2.3	8.0 ± 2.3	9.3 ± 2.2	8.9 ± 2.1
HCV genotype (1/2) (*n* = 135)	60/75 (44.4%/55.6%)	32/35 (47.8%/52.2%)	16/26 (38.1%/61.9%)	12/14 (46.2%/53.8%)
Antiviral treatment				
No antiviral treatment	102 (58.7)	45 (54.2)	25 (53.2)	32 (72.7)
Treatment without SVR	34 (19.5)	14 (16.9)	11 (23.4)	9 (20.5)
Treatment with SVR[b,c]	38 (21.8)	24 (28.9)	11 (23.4)	3 (6.8)

Data are presented as mean ± SD or number (%). [a]*P* value < 0.05 between patients with chronic hepatitis and liver cirrhosis; [b]*P* value < 0.05 between patients with chronic hepatitis and HCC; [c]*P* value < 0.05 between patients with liver cirrhosis and HCC. ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; AFP: Alpha-fetoprotein; INR: International normalized ratio; SVR: Sustained virologic response.

Data collection and patient follow-up

At enrollment, data on the demographics, socioeconomic status, alcohol consumption, and smoking habits were obtained using a standard questionnaire. In addition, laboratory tests, ultrasonography or CT examination of the liver was performed for all patients at baseline. Those collected data were entered into the electronic case report form at the Korean HCV cohort study group homepage (available from URL: http://www.hcvcohort.or.kr/).

All patients were monitored for clinical status and given laboratory tests and imaging examinations, including ultrasonography, CT or MRI, every 3-12 mo. A total of 72 patients were treated with Pegylated interferon alpha (peg-IFNα) and ribavirin for 24-48 wk according to HCV genotype before the study enrollment (*n* = 24) and during the study period (*n* = 48). Survival and mortality, including cause of death, were confirmed using examination of the final medical records, telephone calls to participants or their family members, and death certificate data obtained from the Korean Statistical Information Service[16]. The disease progression was defined as: (1) occurrence of liver cirrhosis, HCC, decompensation (worsening of Child-Pugh class), or mortality in patients with chronic hepatitis; (2) occurrence of HCC, decompensation, or mortality in patients with cirrhosis; and (3) occurrence of mortality in patients with HCC. Time to disease progression was defined as the interval between the date of enrollment and the date of occurrence of HCC, liver cirrhosis, worsening of Child Pugh class, death, last observation, or September 30, 2015.

Blood collection and HBV DNA detection

Serum or plasma samples were obtained from 174 patients and stored at -70 ℃. HBV DNA was extracted from 400 μL of serum or plasma using a QIAamp DNA Blood Mini Kit, (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. DNA was eluted from the spin column in 50 μL of elution buffer. Nested polymerase chain reaction (PCR) was conducted with AccuPower HotStart PCR PreMix (Catalog No. k-5051; Bioneer Inc., Seoul, Republic of Korea) using 4 sets of primers to detect S, C, P and X regions of the HBV genome (Supplementary Table 1). According to the *Taormina Expert Meeting Statements*[17], the presence of OBI was defined as proved positivity in 2 or more different viral genomic regions by nested PCR. AM6 plasmid purchased from the Korea Cell Line Bank (positive control) and serum obtained from normal healthy persons or distilled water (negative control) was used. The first round of PCR was performed in a final volume of 20 μL at 94 ℃ for 2 min followed by 35 cycles of 94 ℃ for 30 s,

52-65 ℃ for 30 s (52 ℃ for S, 58 ℃ for C, 58 ℃ for P, 65 ℃ for X) and 72 ℃ for 30 s, and a final extension step of 72 ℃ for 5 min. Using 5 μL of the first PCR product, the second round PCR was performed under the same conditions as the first round of PCR except for the annealing step temperature (58 ℃ for S, 52 ℃ for C, 52 ℃ for P, 65 ℃ for X). By nested PCR with sets of 10-fold serially diluted AM6 templates, the detection limit was estimated as 54.5 copies/μL of AM6 plasmid.

HBV DNA sequence analyses and genotyping

For 5 OBI-positive samples, the entire S region (the preS1, preS2 and S region) of the HBV genome was amplified by nested PCR using different sets of primers. The PCR products were purified and sequenced to identify the HBV genotype. The condition for the first and second round of PCR was 95 ℃ for 5 min, followed by 30 cycles of 95 ℃ 60 s, 52 ℃ 45 s, and 72 ℃ 90 s with a final extension of 72 ℃ for 5 min. The PCR products were analyzed by electrophoresis on 1% agarose gels, which were stained with Gel Red, and visualized on a UV transilluminator. The PCR products were sequenced by a commercial sequencing company (Bioneer Inc.). HBV DNA sequences were aligned using Clustal W (http://www.clustal.org), and phylogenetic trees were constructed using the neighbor-joining method. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 re-samplings. MEGA version 6.0.6 was used for phylogenetic tree construction and mutation analysis. We determined HBV genotypes by phylogenetic analysis based on 14 reference strains obtained from GenBank (accession numbers AY641558.1, AF286594.1, AY247032.1, AY641559.1, D16667.1, D50519.1, AF305422.1, M57663.2, X70185.1, AB100695.1, D00329.1, AB074755.1, X02496.1, AB554024.1).

Statistical analysis

Categorical variables were compared with the chi-square test or 2-tailed Fisher's exact test, and continuous variables were compared with the Mann-Whitney test or Kruskal-Wallis test. The cumulative probabilities of disease progression and HCC were analyzed using the Kaplan-Meier method. Predictors associated with disease progression and HCC were determined by the Cox proportional regression model. Risk was expressed by hazard ratio and 95%CI. A P value of < 0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using PASW software (version 22; IBM SPSS Statistics for Windows, Armonk, NY, United States).

RESULTS

Patient characteristics

The baseline characteristics of the 174 study patients with chronic HCV infection are summarized in Table 1, showing the mean age to be 66.5 years, male sex for 60.3%, and past or current alcohol users of 46.2%. They included 83 patients with chronic hepatitis, 47 patients with liver cirrhosis and 44 patients with HCC. The HCV genotype 1 and 2 were detected in 60 patients (44.4%) and 75 patients (55.6%), respectively. Antiviral therapy with peg-IFNα and ribavirin combination regimen was undertaken in 72 patients before enrollment or during follow-up, and overall sustained virologic response (SVR) rate (defined as undetectable serum HCV RNA at 24 wk after the end of the treatment) was 52.8%. The anti-hepatitis B core immunoglobulin G test was performed in 100 patients, for which 75 patients showed positive results.

Prevalence of OBI and clinical factors related to OBI positivity

The positive detection rate of plasma or serum HBV DNA was 18.4% (32 among 174 total patients) defined as at least 2 positive results on nested PCR among 4 different sets covering 4 ORFs of HBV genomes. The positive rate was not different among 3 different diagnostic categories at enrollment: 14 of 83 (16.9%) in chronic hepatitis C, 12 of 47 (25.5%) in liver cirrhosis, and 6 of 44 (13.6%) in HCC (Figure 1). Therefore, the prevalence of OBI did not parallel the severity of liver disease at study enrollment.

To investigate the clinical factors that might be associated with OBI positivity, we compared various variables including age, sex, body mass index, laboratory results, model for end-stage liver disease score, Child-Pugh score and HCV genotypes between the patients with and without OBI. However, there were no significant differences between OBI-positive and OBI-negative patients, as shown in Table 2.

Effect of OBI positivity on disease progression and HCC development

During the mean follow-up duration of 37.4 mo, 52 patients showed composite disease progression: 12 patients developed liver cirrhosis from chronic hepatitis; 13 patients developed decompensated cirrhosis (worsening of Child-Pugh class); 14 patients developed HCC; and 13 patients died. As shown in Table 3, OBI positivity was not a significant factor associated with disease progression in either univariate or multivariate analysis. However, AST > 40 IU/L, increased Child-Pugh score at enrollment, and SVR were significantly associated with disease progression in multivariate analysis. Moreover, there was no significant difference in development of disease progression between the patients with or without OBI (Figure 2A).

Of the 130 patients without HCC at enrollment, there was no significant difference in HCC development between the patients with or without OBI (Figure 2B). On multivariate analysis, Child-Pugh score was an independent factor for HCC development (Table 4).

After exclusion of 34 patients who achieved SVR

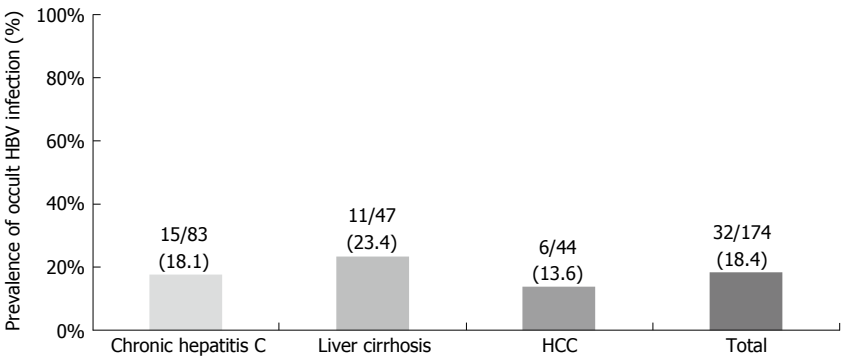

Figure 1 At study enrollment, the prevalence of occult hepatitis B virus infection in blood of patients with chronic hepatitis C virus infection. The prevalence of HBV DNA in blood among 174 patients was 32 (18.4%) by nested PCR; 15 of 83 (18.1%) were positive in the chronic hepatitis C group, 11 of 47 (23.4%) in the liver cirrhosis group, and 6 of 44 (13.6%) in the HCC group. The difference was not significant. HCC: Hepatocellular carcinoma.

Table 2 Comparison of clinical factors between patients with and without occult hepatitis B virus infection

Variable	OBI (+) (*n* = 32)	OBI (-) (*n* = 142)	*P* value
Mean age, yr	67.1 ± 9.6	66.4 ± 9.9	0.952
Male sex	19 (59.4)	86 (60.6)	0.527
Body mass index (kg/m^2)	23.9 ± 3.2	23.2 ± 3.0	0.180
Anti-HBc (*n* = 100)	6 (66.7)	69 (75.8)	0.399
Anti-HBs (*n* = 157)	14 (48.3)	72 (56.3)	0.283
Hemoglobin (g/mL)	13.3 ± 1.3	13.5 ± 1.9	0.364
Platelet (× 10^3/mL)	150.5 ± 79.5	151.5 ± 89.3	0.978
Albumin (g/dL)	3.9 ± 0.5	4.0 ± 0.4	0.593
Total bilirubin (mg/dL)	1.1 ± 0.8	1.0 ± 0.5	0.118
AST (IU/L)	62.7 ± 40.3	78.8 ± 112.6	0.546
ALT (IU/L)	54.7 ± 43.5	82.2 ± 151.6	0.148
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.4	0.779
PT-INR	1.0 ± 0.1	1.0 ± 0.1	0.459
MELD score	8.3 ± 1.9	8.6 ± 2.4	0.744
HCV genotype (1/2)	12/15 (44.4%/55.6%)	48/60 (44.4%/55.6%)	0.587
AFP > 20 ng/mL	6 (19.4)	23 (16.5)	0.441
Child-Pugh class			0.436
A	28 (87.5)	132 (93.0)	
B	4 (12.5)	8 (5.6)	
C	0	2 (1.3)	
Antiviral treatment			
No antiviral treatment	21 (65.6)	81 (57.0)	0.430
Treatment without SVR	5 (19.2)	29 (26.4)	0.616
Treatment with SVR	6 (18.8)	32 (22.5)	0.814
Disease progression	7 (21.9)	45 (31.7)	0.392
Development of HCC	3 (11.5)	11 (10.6)	1.000
Follow-up period (mo)	42.5 ± 34.7	36.3 ± 26.9	0.555

Data are presented as mean ± SD or number (%). OBI: Occult hepatitis B virus infection; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; AFP: Alpha-fetoprotein; PT-INR: Prothrombin time-international normalized ratio; SVR: Sustained virologic response.

during the study period, OBI was not a significant factor associated with either disease progression, nor HCC development (Supplementary Figure 1).

OBI positivity according to serological markers of HBV

Anti-HBs and anti-HBc were both evaluated in 87 patients. OBI positivity was 2.9% (1/34) in anti-HBc (+) and anti-HBs (+) patients, 9.4% (3/32) in anti-HBc (+) and anti-HBs (-) patients, 11.1% in anti-HBc (-) and anti-HBs (+) patients, and 15.4% in anti-HBc (-) and anti-HBs (-) patients. Therefore, positivity for anti-HBc alone did not represent OBI positivity.

HBV DNA genotype of HBV strains in OBI-positive patients

In the 32 OBI-positive patients, 5 samples were available for full S-ORF sequence analysis including preS1, preS2 and S gene. The phylogenetic analysis showed that all 5 samples were HBV genotype C2, which was same as the HBV genotype reported in almost all patients with chronic HBV infection in South Korea.

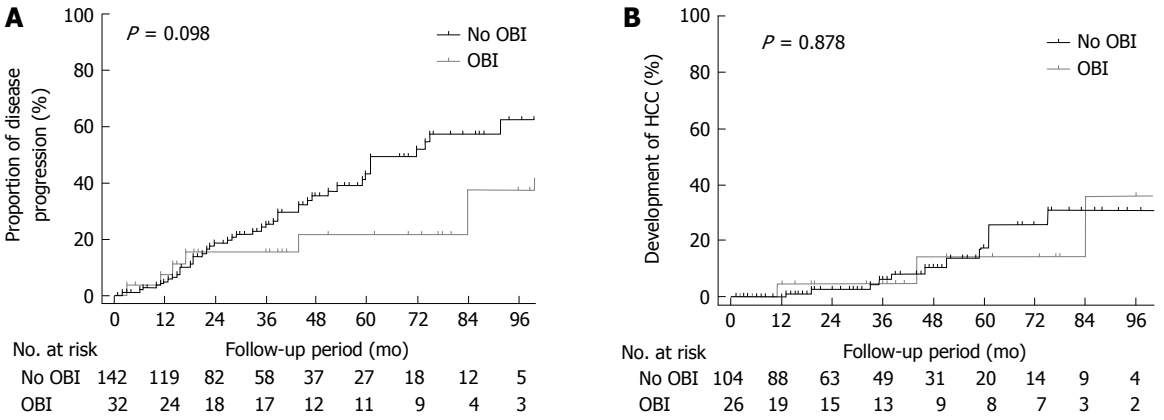

Figure 2 Effect of occult hepatitis B virus infection positivity on disease progression and hepatocellular carcinoma development. A: Cumulative incidence of disease progression defined as development of liver cirrhosis or HCC, worsening of Child Pugh class (A→B or C), or mortality according to OBI; B: Cumulative incidence of development of HCC according to OBI. HCC: Hepatocellular carcinoma; OBI: Occult hepatitis B virus infection.

Table 3 Clinical factors associated with disease progression, defined as development of liver cirrhosis, decompensation, hepatocellular carcinoma, or mortality (n = 174)

Variable	HR	*P* value	Adjusted HR	*P* value
OBI	0.494 (0.210-1.164)	0.107	0.510 (0.208-1.251)	0.141
Age (per year)	1.020 (0.990-1.050)	0.194		
Male sex	0.935 (0.534-1.636)	0.814		
Anti-HBc positivity	2.291 (0.878-5.976)	0.090		
AST > 40 IU/L	3.730 (1.740-7.996)	0.001	3.419 (1.117-10.463)	0.031
ALT > 40 IU/L	2.454 (1.273-4.730)	0.007	0.737 (0.297-1.826)	0.510
GGT > 70 IU/L	2.736 (1.571-4.765)	< 0.001		
AFP > 20 ng/mL	2.247 (1.238-4.079)	0.008	1.370 (0.721-2.600)	0.336
Child-Pugh score (per unit)	2.136 (1.628-2.802)	< 0.001	1.716 (1.230-2.394)	0.001
MELD score (per unit)	1.111 (1.009-1.224)	0.032	0.987 (0.860-1.134)	0.856
SVR	0.263 (0.104-0.663)	0.005	0.317 (0.121-0.828)	0.019
HCV genotype 1	1.465 (0.780-2.753)	0.231		

Disease progression defined as: (1) occurrence of liver cirrhosis, hepatocellular carcinoma (HCC), decompensation, or mortality in patients with chronic hepatitis; (2) occurrence of HCC, decompensation, or mortality in patients with cirrhosis; and (3) occurrence of mortality in patients with HCC. HR: Hazard ratio; OBI: Occult hepatitis B virus infection; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: Alpha-fetoprotein; MELD: Model for end-stage liver disease; SVR: Sustained virologic response; HCV: Hepatitis C virus.

DISCUSSION

In this study, the prevalence of OBI was 18.4% in the blood of HBsAg-negative patients with chronic HCV infection in South Korea by nested PCR, in whom OBI prevalence was not higher in the HCC group (13.6%) than in the chronic hepatitis group (18.1%) or liver cirrhosis group (23.4%). Moreover, neither positive OBI nor presence of anti-HBc was significantly associated with disease progression or HCC development on multivariate analysis. The HBV genotype detected in all 5 OBI-positive patients was genotype C2, which was the same as almost all of the detected genotypes in chronic hepatitis B patients in Korea.

The prevalence of OBI in the liver tissue or serum of anti-HCV-positive patients has been reported variously, both in prospective and retrospective studies. The prevalence of OBI detected in liver tissue was reported as 38.8% in 326 Italian patients with chronic HCV infection[18], 57% in 100 Portuguese patients[19], and 15.0% in 167 Japanese patients as determined by nested PCR[13], and 50% in 44 patients with HCV-related advanced cirrhosis in the United States as determined by real-time PCR[20].

The prevalence of OBI in serum/plasma in HCV-related liver disease patients was 5.6% (8/141)[21], 7.8% (11/140)[22], and 5.2% (9/173) as determined by real-time PCR in Japan[23], but it was 43.6% (204/468) in one Japanese study that using nested PCR with only one set of primers covering the X region[24]. The OBI prevalence in chronic HCV patients was 45.7% (42/92) in Morocco[25] and 20% (18/50) in Iran[26], while none of 100 Portuguese patients showed serum OBI[19]. A retrospective study in Taiwan showed that serum OBI prevalence as determined by nested PCR using 3 sets of primers in patients with chronic HCV infection was 14.8% (31 of 210), which did not differ from that of healthy controls (15%, 15/100), and the prevalence of

Table 4 Clinical factors associated with development of hepatocellular carcinoma

Variable	HR	*P* value	Adjusted HR	*P* value
OBI	0.904 (0.251-3.264)	0.878	0.860 (0.209-3.535)	0.835
Age (per year)	1.016 (0.962-1.073)	0.571		
Male sex	1.531 (0.536-4.374)	0.427		
AST > 40 IU/L	6.120 (1.351-27.729)	0.019	3.383 (0.664-17.226)	0.142
ALT > 40 IU/L	3.573 (0.976-13.075)	0.054		
Creatinine (per unit)	0.051 (0.003-0.892)	0.042	0.075 (0.003-2.200)	0.133
AFP > 20 ng/mL	3.381 (1.129-10.121)	0.029	1.706 (0.512-5.678)	0.384
PT-INR (per unit)	10.081 (1.036-77.802)	0.027		
Child-Pugh score (per unit)	3.065 (1.741-5.399)	< 0.001	2.818 (1.547-5.135)	0.001
MELD score (per unit)	1.057 (0.862-1.296)	0.597		
SVR	0.289 (0.064-1.302)	0.106		
HCV genotype 1	2.049 (0.644-6.517)	0.224		

HR: Hazard ratio; OBI: Occult hepatitis B virus infection; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: Alpha-fetoprotein; PT-INR: Prothrombin time-international normalized ratio; MELD: Model for end-stage liver disease; SVR: Sustained virologic response; HCV: Hepatitis C virus.

OBI did not parallel the severity of liver disease (14.5% in chronic hepatitis, 8% in liver cirrhosis, and 22% in HCC)[27]. In the present study, the prevalence of OBI in plasma was 18.4% (32 of 174), which did not increase with the severity of liver disease, showing similar results to the Taiwan study consisting of the subjects and detection methods similar to this study.

The variously reported OBI prevalence in patients with chronic HCV infection may be related to the different study subjects, different materials used (liver tissue *vs* serum), or differences in PCR technology such as nested PCR or real-time PCR using different numbers of primer sets. Our method followed the *Taormina Expert Meeting Statements*[17], in which the presence of OBI was defined as proved positivity in 2 or more different viral genomic regions by nested PCR and included adequate positive and negative controls. Previous data suggested that the prevalences of OBI detected in liver tissue were higher than those in blood, though reports of head-to-head comparisons of both methodologies are limited[19,28]. Integration of the HBV genome into the host genome is a plausible explanation. In addition, risk factors of HCV infection may affect the prevalence of OBI. As shown in the above United States study, a history of intravenous drug abuse (IVDU) was found in 66% of the subjects, as opposed to 1% of our study population. Because HBV and HCV can share the parenteral transmission route, repeated exposure to HBV or HCV during IVDU may result in relatively high frequency of OBI in those subjects rather than those who were infected through perinatal infection as in Korea.

This study showed that OBI prevalence did not increase according to the severity of liver disease (n = 174), and during a mean prospective follow-up of 38 mo, OBI was not associated with either HCC development or the overall disease progression. However, SVR, Child-Pugh score and AST level were independent factors associated with the disease progression. Even in the patients who did not receive treatment or did not achieve SVR (n = 136), OBI positivity was not associated with either HCC development or disease progression.

The role of OBI in disease progression or development of HCC in patients with chronic HCV infection is still a matter of considerable controversy. Some studies have reported that OBI may contribute to the development of HCC or cirrhosis in chronic HCV-infected patients[9,10,18,21,23,24,29]. In contrast, other reports have shown that OBI is not an important factor in the progression to HCC or cirrhosis[11,12,14,27,28,30,31]. Most previous studies were cross-sectional studies[12,16,18,24], and a meta-analysis including both prospective and retrospective studies reported that OBI contributed to the development of HCC[32]. A prospective study in Italy showed that among 94 patients who were tested for liver OBI and followed for a median 11 years, HCC developed more often in the OBI-positive group (13/37) than in the OBI-negative group (5/57), and OBI-positive patients had shorter cumulative survival rate than OBI-negative patients. Though this study suggests that OBI may lead to HCC development and lower survival, only 94 among a total 326 original study group were followed (follow-up missing rate of 71.2%) and 79 out of 94 patients underwent antiviral treatment with an SVR rate of 33% (26/79). Considering that SVR is a strong independent factor for HCC development or survival, more studies are needed to clarify the significance of OBI in HCV-related liver disease progression.

The possible mechanisms of OBI involvement in hepatocarcinogenesis were HBV-induced accelerated inflammation, HBV genome integration in the host DNA, or promoting effect of HBV proteins in malignant transformation. In a woodchuck model, persistently low level of viremia in liver tissue in the absence of woodchuck HBsAg can lead to liver injury and HCC development[33]. However, there has been no clear

evidence supporting the hepatocarcinogenic role of OBI in HCV-related liver disease. Two recently reported independent *in vitro* studies showed that both HBV and HCV can replicate in the same hepatocyte cells without evidence of viral interference. Therefore, HCV and HBV may interfere with each other by indirect effects of host-viral interactions or host immune response.

Moreover, the epidemiology of HCV or HBV is different according to geographic regions or population. For example, the most common mode of transmission of HBV in Korean people has been perinatal transmission, while HCV infection occurs in later life. In contrast, IVDUs in an HBV non-endemic area may be infected by both viruses simultaneously or repeatedly. Those differences in epidemiology may lead to differences in immunological response to OBI in HCV-infected patients in different regions. In this study, a phylogenetic analysis showed that 5 strains from the occult HBV-infected subjects were all genotype C2, which was detected in nearly 100% of chronic hepatitis B patients in Korea. Because of sample availability, only 5 samples among the 32 patients with OBI were evaluated. Several mechanisms have been considered for occult infections by HBV, such as low HBV DNA and HBsAg levels, mutations in HBV DNA sequence, viral DNA integration in the host genome, infection of peripheral blood mononuclear cells, production of immune complexes containing HBV, altered host immune response, and interference of HCV[34-37].

The present study had several limitations, including being a single center study with relatively small sample size, no availability of liver tissue and no results on quantitative evaluation of HBV DNA.

In conclusion, this study demonstrates that the prevalence of OBI in blood in patients with chronic HCV infection in South Korea was 18.4%, with no significant correlation between OBI positivity and disease progression or HCC. Controversy still exists regarding the role of OBI; therefore, well-designed prospective multicenter studies and experimental studies are warranted.

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COMMENTS

Background

Whether the occult hepatitis B virus (HBV) infection (OBI) affects the outcomes of chronic hepatitis C virus (HCV) infection is controversial. This study aimed to clarify the prevalence of OBI and the association between OBI and liver disease progression in cases of chronic HCV infection.

Research frontiers

The prevalence of OBI in chronic HCV-infected patients has been reported variously in different global regions. The pathogenic role of OBI in the disease progression or development of hepatocellular carcinoma (HCC) in patients with chronic HCV infection is still a matter of considerable controversy. There are no data on the prevalence or effect of OBI in chronic HCV infection in Korea, an HBV endemic region.

Innovations and breakthroughs

The positive detection rate of plasma or serum HBV DNA was 18.4% (32 among 174 patients), defined as at least 2 positive results on nested polymerase chain reaction (PCR) using 4 sets of primers in the S, C, P and X open reading frame of the HBV genome. However, OBI positivity was not a significant factor associated with disease progression. In addition, there was no significant difference in HCC development between the patients with or without OBI.

Applications

This study demonstrates that the prevalence of OBI in patients with chronic HCV infection in Korea was 18.4%, with no significant correlation between OBI positivity and disease progression or HCC. Controversy still exists regarding the role of OBI; therefore, well-designed prospective multicenter studies and experimental studies are warranted.

Terminology

According to the *Taormina Expert Meeting Statements,* the presence of OBI was defined as proved positivity in 2 or more different viral genomic regions by nested PCR using 4 sets of primers to detect the S, C, P and X regions of the HBV genome. The disease progression was defined as: (1) occurrence of liver cirrhosis, HCC, decompensation (worsening of Child-Pugh class), or mortality in patients with chronic hepatitis; (2) occurrence of HCC, decompensation, or mortality in patients with cirrhosis; and (3) occurrence of mortality in patients with HCC.

Peer-review

This observational study represents an unbiased and well-articulated report on the usefulness of OBI measurement as a prognostic factor for liver disease progression.

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