

***Helicobacter pylori* morbidity in chronic hepatitis B patients: A case-control study.**

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Abstract

We conducted a case-control study performed in the China-Japan Union Hospital of Jilin University, China from 2010 to 2015 to investigate the seroprevalence of *Helicobacter pylori* infection in patients with chronic hepatitis B. The cases were 853 patients with chronic hepatitis B who were sex and age matched to 729 healthy controls. All subjects were tested for the presence of serum anti-*H. pylori*-IgG. *H. pylori* infection was more prevalent in patients with chronic hepatitis B (59.6%), chronic Hepatitis B virus (HBV)-related cirrhosis (77.3%), and Hepatocellular Carcinoma (HCC) (80.3%) than in healthy controls (43.3%) ($\chi^2=35.708$, $\chi^2=53.175$, $\chi^2=29.501$, respectively; $P<0.05$). The differences in *H. pylori* prevalence among the case groups by the serum viral load of HBV DNA were not statistically significant ($P>0.05$). No significant difference among groups by genotype was detected ($P>0.05$). *H. pylori* infection was more highly prevalent in cirrhosis patients with complications such as hepatic encephalopathy (69.6%), peptic ulcers (61.0%), and upper gastrointestinal hemorrhage (78.7%) than in patients without complications ($\chi^2=7.713$, $\chi^2=4.293$, $\chi^2=16.517$, respectively; $P<0.05$). Whether *H. pylori* act synergistically with HBV for the progression from chronic hepatitis B to cirrhosis and HCC requires further research.

Keywords: *Helicobacter pylori* (*H. pylori*), Hepatitis B virus (HBV), Chronic hepatitis B (CHB), HBV-related cirrhosis, Hepatocellular carcinoma (HCC), HBV DNA.

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Introduction

Hepatitis B Virus (HBV) infection is a primary etiological factor of chronic hepatitis, cirrhosis, and Hepatocellular Carcinoma (HCC) [1,2]. However, only a subset of patients infected by HBV develops chronic hepatitis, cirrhosis, and HCC despite being infected with the same genotype of HBV and having similar genetic backgrounds. The mechanism triggering progression of the disease remains unclear. Researchers are actively investigating whether other co-factors, such as bacterial infections, have effects on its progression.

Fox et al. [3] and Ward et al. [4] reported that *Helicobacter hepaticus* could induce hepatitis leading to HCC. Although *H. hepaticus* differs from *Helicobacter pylori* in ultramicrostructure and biologic characteristics, they belong to the same *Helicobacter* genus. *H. pylori* infection is widespread with a seroprevalence of about 50% in the general population [5]. Epidemiology studies have shown that *H. pylori*

prevalence is higher in patients with hepatitis B, cirrhosis, and HCC than in healthy blood donors [6-8].

There is a high rate of HBV infection in China [9-11], but there are no prior epidemiology reports about *H. pylori* prevalence in Chronic Hepatitis B (CHB) patients in Jilin Province. We performed a hospital-based case-control study to verify *H. pylori* seroprevalence in 853 CHB patients to investigate the interaction of *H. pylori* and HBV in CHB.

Materials and Methods

Patients

This study was a case-control study performed in China-Japan Union Hospital of Jilin University, China. The inclusion criteria for the cases were: 1) age ≥ 16 y; 2) diagnosed as hepatitis B surface antigen (HBsAg) positive by Enzyme-Linked Immunosorbent Assay (ELISA) detection used

commercial kits; and 3) expected lifespan was more than 5 y. Exclusion criteria were: 1) malignancy except for HCC; and 2) pregnant or <16 y old.

The presence of anti-*H. pylori* antibodies was confirmed in blood samples from 853 CHB patients who were in and outpatients of China-Japan Union Hospital of Jilin University (657 men, 196 women, aged from 16 to 66 y, mean age 35.3 ± 15.7) from 2010 to 2015. Among these patients, 651 had CHB (519 men, 132 women), 141 had HBV-related cirrhosis (95 men, 46 women), and 61 had HCC (43 men, 18 women). All of these patients were diagnosed according to the 2010 Chronic Hepatitis B Prevention and Cure Manual of China. Other factors affecting liver disease, such as HCV infection, autoimmune diseases, metabolic diseases, and alcohol intake, were ruled out.

The controls were 729 sex and age-matched blood donors (515 men, 214 women, aged from 21 to 60 y, mean age 36.6 ± 14.5 y) during the same period. No subjects (cases and controls) underwent antibiotic therapy for at least 6 months before entry into the study. This study was conducted in accordance with the declaration of Helsinki and approval from the Ethics Committee of China-Japan Union Hospital of Jilin University. Written informed consent was obtained from each participant.

Assessment of *H. pylori* infection

A commercial ELISA assay (Huamei Biotechnology Company, Hangzhou, China) was used to detect anti-*H. pylori*-IgG in serum. Briefly, 100 μ L of diluted (1:100) serum samples, positive controls, and negative controls were added to wells coated with purified *H. pylori* group-specific antigens and mixed sufficiently. The plates were incubated for 30 min at 37°C. After incubation, the liquid was removed completely and the wells were washed five times with 200 μ L per well of washing solution; the liquid was removed and 100 μ L of anti-IgG conjugate was pipetted into each well and the wells were incubated for 30 min at 37°C. The washing step was repeated and then 50 μ L of chromogenic substrates A and B were added to each well and incubated for 10 min at 37°C away from light; the reaction was stopped by adding 50 μ L of stop solution. Reading was performed at 450 nm after setting the spectrophotometer to zero on the blank well and then the Optical Density (OD) of each well was read. A negative control OD lower than 0.05 was expressed as 0.05, and any reading higher than 0.05 was expressed as the real OD. The sample was defined as anti-*H. pylori*-IgG positive if the samples' OD/the negative control was ≥ 2.1 .

Quantification of HBV DNA

The serum HBV DNA was quantitated by real-time PCR using an HBV diagnostic kit (DA Biotech, LTD, Guangzhou, China). The assay was performed strictly in accordance with the manufacturer's instructions. The lowest level of detection of the kit is 1.0×10^2 IU/ml.

Genotypes of HBV DNA

A commercial assay for the genotypes of serum HBV DNA, the HBV DNA genotypes chain reaction kit (PCR-BLOT-ELISA) (DA Biotech, LTD, Guangzhou, China), was used. The assay was performed strictly in accordance with the manufacturer's instructions.

Statistical analysis

Student's t test was used to compare the potential differences among the groups, and a chi-square test (χ^2) was used to compare the ratios. Fisher's exact test was used when there was a small sample size. Results were defined as significant when $P < 0.05$.

Results

***H. pylori* seroprevalence in chronic hepatitis B patients**

H. pylori prevalence in CHB patients (63.9%) was higher than in the healthy donors (43.3%) ($P < 0.01$, $\chi^2 = 66.064$, OR 2.313, 95% CI 1.889-2.832). In order to exclude the influence of pathological changes, the cases were divided into three groups (CHB, cirrhosis, and HCC). *H. pylori* prevalence in CHB patients (59.6%), HBV related cirrhosis patients (77.3%), and HCC patients (80.3%) were all higher than in healthy donors ($P < 0.05$), and *H. pylori* prevalence in HBV related cirrhosis patients and HCC patients was clearly higher than that in CHB patients (Table 1).

Association between *H. pylori* infection and the quantitation of HBV DNA

The 853 CHB patients were divided into different groups according to the serum viral load of HBV DNA, and *H. pylori* prevalence in these different groups was calculated. *H. pylori* seroprevalences in the different groups of HBV DNA (+) patients were higher than in HBV DNA (-) patients and healthy controls, but no significant differences among the HBV DNA (+) groups were detected (Table 2).

Association between *H. pylori* infection and the genotypes of HBV

Among the 853 chronic hepatitis patients, we examined the genotype of HBV in 185 patients who were HBV DNA (+) and analysed the association between *H. pylori* infection and the genotypes of HBV. Type C (58.4%) was the most common genotype. The seroprevalence of *H. pylori* was 40.0% (2/5) for type A, 50.9% (27/53) for type B, 50.9% (55/108) for type C, and 52.6% (10/19) for type D. No significant difference was found among the types ($P > 0.05$).

Association between *H. pylori* infection and the complications of HBV-related cirrhosis

Among the 141 chronic HBV-related cirrhosis patients, 46 patients suffered from hepatic encephalopathy and 32 of them (69.6%) were infected by *H. pylori*; 59 patients suffered upper gastrointestinal hemorrhage and 36 of them (61.0%) were infected by *H. pylori*; 61 patients suffered from peptic ulcers

and 48 of them (78.7%) were infected by *H. pylori*. *H. pylori* infection was clearly more prevalent in cirrhosis patients with complications than in patients without complications (39.6%, 21/53) ($\chi^2=7.713$, OR=3.483, 95% CI 1.511-8.030; $\chi^2=4.293$, OR=2.385, 95% CI 1.116-5.098; $\chi^2=16.517$, OR=5.626, 95% CI 2.486-12.825; P<0.05) (Table 3).

Table 1. *H. pylori* seroprevalence in chronic hepatitis B patients.

Group	Patients (n)	<i>H. pylori</i> infection	<i>H. pylori</i> seroprevalence (%)
Chronic hepatitis	853	545	63.9*
CHB	651	388	59.6*
Cirrhosis	141	109	77.3*▲
HCC	61	49	80.3*▲
Healthy donors	729	316	43.3

*Compared with healthy donors P<0.05, ▲Compared with CHB patients and chronic gastritis patients P<0.001.

Table 2. Association between quantitation of HBV DNA and *H. pylori* infection in chronic hepatitis B patients.

	Patients (n)	<i>H. pylori</i> infection	<i>H. pylori</i> seroprevalence (%)
Healthy donors	729	316	43.3
HBV-DNA (-)	214	108	50.5
HBV-DNA (+)	639	437	68.4*▲
10 ² -10 ⁵ IU/ml	183	119	65.0*▲
10 ⁵ -10 ⁷ IU/ml	231	160	69.3*▲
>10 ⁷ IU/ml	225	158	70.2*▲

*Compared with HBV DNA (-) group P<0.05, ▲Compared with healthy donors P<0.001.

Table 3. Association between *H. pylori* infection and the complications of HBV-related cirrhosis.

Group	Patients (n)	<i>H. pylori</i> infection	<i>H. pylori</i> seroprevalence (%)	
Complications	Hepatic encephalopathy	46	32	69.6*
	Upper gastrointestinal hemorrhage	59	36	61.0*
	Peptic ulcer	61	48	78.7*
Without complications	53	21	39.6	

*Compared without complications CHB patients P<0.05.

Discussion

HBV infection is one of the most important causes of chronic hepatitis, cirrhosis, and HCC [1,2]. However, among patients infected with HBV, only a minority of them progress to chronic hepatitis, cirrhosis, and HCC. The mechanism triggering progression is not yet clear. Synergism with other factors, such as bacterial infections, is postulated. Recently, epidemiologic studies have shown that *H. pylori* seroprevalence among

patients with CHB, cirrhosis, and HCC is higher than that of healthy blood donors [6-8].

In our hospital-based case-control study, the *H. pylori* infection status of 853 CHB patients was examined. Our results showed that *H. pylori* seroprevalence was significantly higher than in healthy controls (P<0.05). We also found that the prevalence of *H. pylori* infection in patients with HBV-related cirrhosis and HCC was significantly higher than in CHB patients. Our

results are consistent with the research of Ponzetto et al., Leone et al., and Fan et al. [6-8].

H. pylori might exhibit synergism with HBV in the progression from CHB to cirrhosis and HCC and aggravated liver lesions. In addition, we found that the prevalence of *H. pylori* infection of patients in the HBV DNA (+) group was higher than that in the HBV DNA (-) group ($P < 0.05$) and healthy controls ($P < 0.001$), but there was no significant difference among the viral load groups. This implies that HBV replication is more active in patients with *H. pylori* infections, and this may be associated with *H. pylori* infection down-regulating the immunity of the host.

In this study, *H. pylori* infection in patients with chronic hepatitis showed the characteristics of chronic inflammation. The infiltration of lymphocytes, monocytes, and plasma cells was considered highly suggestive of a host immune reaction to *H. pylori* infection. In immune-compromised liver cells, HBV is particularly difficult to be eradicated by host immunization [12-14]. Unfortunately, we did not identify any association between the genotypes of HBV and *H. pylori* infection. Moreover, *H. pylori* infected patients with chronic liver diseases are prone to suffer complications, especially hepatic encephalopathy, peptic ulcers, and gastrointestinal bleeding [15-20]. Our study found that the prevalence of *H. pylori* infection was higher in patients with HBV-related cirrhosis, and this may play a role in why some patients with cirrhosis suffer from complications.

H. pylori is an important pathogenic factor in peptic ulcers, chronic gastritis, lymphoma gastric mucosa associated lymphoid tissue lymphoma, and gastric carcinoma [21,22]. Recently, *H. pylori* were also shown to be related to many extragastrointestinal diseases, such as iron deficiency anemia, Idiopathic Thrombocytopenic Purpura (ITP), coronary artery disease, and chronic liver disease [23-26]. In patients with chronic liver diseases, especially cirrhosis, the patient is in a state of immune tolerance or immune subversion, and the ability to resist infection of the gastric mucosa is decreased. Local tissue ischemia, hypoxia, and microcirculation disturbances render the tissue prone to being infected by *H. pylori*, which induces gastric mucosal erosion. Meanwhile, because of changes in intestinal flora, the elimination of *H. pylori* is difficult, and therefore *H. pylori* infection is common. Moreover, *H. pylori* can also reach the liver through the blood circulation, and *H. pylori*'s cytotoxic effect can aggravate liver damage.

Herein, the sample size is large enough to support and generalize our conclusions. We compared the morbidity of *H. pylori* between HBV infected and non-infected patients, patients with different clinical symptoms, different HBV DNA loads, and different HBV genotypes.

Due to the study's case-control design, the predictive value of *H. pylori* infection in CHB patients remains clear. Because of the poor long-term prognosis of chronic infection of HBV and *H. pylori*, the former finally leads to HCC. However, the role of *H. pylori* infection may be at an early stage, namely to cause

chronic inflammation. Whether *H. pylori* act synergistically with HBV in the progression from chronic hepatitis B to cirrhosis and HCC needs further research.

Conclusion

H. pylori seroprevalence was higher in patients with chronic hepatitis B than in healthy controls. Whether *H. pylori* act synergistically with HBV in the progression from chronic hepatitis B to cirrhosis and HCC needs further research.

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Conflict of Interest

All authors have no conflict of interest regarding this paper.

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