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Hepatitis B virus infection is associated with gastric cancer in China: an endemic area of both diseases

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Background: Hepatitis B virus (HBV) infection was demonstrated to be a risk factor of several cancers of the digestive system. In addition, liver cirrhosis, which could possibly result from chronic HBV infection, was associated with a higher risk of gastric cancer. However, the association of HBV infection and gastric cancer has not been investigated.

Methods: A retrospective case–control study with 580 cases and 580 controls matched for age, sex and year of diagnosis was conducted. The associations between gastric cancer and HBV infection were explored with univariate and multivariate unconditional logistic regression analysis.

Results: Hepatitis B surface antigen (HBsAg) was positively associated with gastric cancer (AOR (95% CI): 1.49 (1.06–2.10)). This association remained significant in patients without family history of gastric cancer (AOR (95% CI): (1.06–2.11)). For HBsAg-negative population, being anti-HBc positive/anti-HBs negative, which possibly indicated occult HBV infection, was also found to have some associations with gastric cancer. In addition, some synergistic effects between HBV infection and blood type A in gastric cancer were identified.

Conclusions: The HBV infection was positively related with gastric cancer, especially for patients without family history of gastric cancer. Further prospective studies are warranted to confirm this relationship.

Gastric cancer (GC) is one of the most common malignant diseases in the digestive system, contributing to ~10% of annual deaths from cancer. Although the incidence of GC has been declined for several decades, it is still the fourth most common cancer in men and fifth in women (Jemal *et al*, 2011). Several risk factors have been explored. The most thoroughly investigated and commonly recognised factor is the infection of *Helicobacter pylori* (Hp) (Correa *et al*, 1990; Niwa *et al*, 2010). Other factors including family history of GC (Yaghoobi *et al*, 2010), genetic factors (Gonzalez *et al*, 2002; Loh *et al*, 2009), excessive salt intake (Peleteiro *et al*, 2011), alcohol drinking (Tramacere *et al*, 2012), smoking (Ladeiras-Lopes *et al*, 2008) diabetes mellitus (Yoon *et al*, 2013) and blood type A (Wang *et al*, 2012) are also quite convincing, whereas the role of Epstein–Barr virus (EBV) infection (Kim *et al*, 2009; Yuan *et al*, 2013) as well as lack of fruits and vegetables consumption (Norat *et al*, 2014) is controversial. Beyond these, opium is also proposed to be a risk factor for gastric adenocarcinoma recently (Shakeri *et al*, 2013; Sadjadi *et al*, 2014).

The prevalence of hepatitis B virus (HBV) infection varies largely worldwide. The relatively high prevalence areas are located in some developing countries, including China (Dehesa-Violante

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and Nunez-Nateras, 2007). The HBV infection was once a major health burden in China, but the prevalence rate has dropped steadily from no less than 8% to 2–7% in recent years since the application of universal infant immunisation policy (Lu *et al*, 2010). Hepatitis B virus is considered to be a hepatotrophic virus, and it has long been confirmed to be the most important risk factor for hepatocellular carcinoma (Yu *et al*, 1990). However, several studies have probed the existence of HBV in some extrahepatic organs and tissues, such as the kidneys, skin, lymph nodes, bone marrow, vessel walls, colon, pancreas as well as stomach (Dejean *et al*, 1984; Mason *et al*, 1993; Chen *et al*, 2004). Moreover, it has been put forward that HBV infection is also a risk factor for non-Hodgkin's lymphoma (Engels *et al*, 2010) and pancreatic cancer (Hassan *et al*, 2008).

In a study by Chen *et al* (2004), the coexistence of Hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) with Hp immunoglobulin G antigen in gastric antrum mucosa was observed in patients with chronic HBV infection or HBV-related cirrhosis. In addition, they found that there was no difference in the rates of HBV antigen expression between the Hp- positive and -negative patients. It was also found that patients with liver cirrhosis had a high prevalence of gastric ulcers (Kirchner *et al*, 2011) and an increased risk of GC (Zullo *et al*, 2003). Besides, the impact of Hp on gastric ulcers in patients with liver cirrhosis was found to be relatively weak (Kirchner *et al*, 2011). It is well known that HBV infection is the most important risk factor for liver cirrhosis in China. We therefore speculate that HBV infection may play a role in the risk of GC in China, as China is an endemic region for both GC and HBV infection. However, the markers of either past or present HBV infection could possibly be also frequently detectable in patients with GC. The relation between HBV infection and GC might be a casual association. Thus, we launched this case–control study to investigate the associations of HBV infection with GC.

MATERIALS AND METHODS

Ethics statement. All patients provided written informed consent for their information to be stored and used in the hospital database. Study approval was obtained from independent ethics committees at Cancer Center of Sun Yat-sen University. The study was undertaken in accordance with the ethical standards of the World Medical Association Declaration of Helsinki.

Study population. There were 725 patients potentially diagnosed with GC from 1 September 2007 to 31 December 2009 at Sun Yat-sen University Cancer Center in Guangzhou, China. Patients pathologically diagnosed with other types of cancer such as gastric neuroendocrine tumour, gastric stromal tumour and gastric mucosa-associated lymphoid tissue lymphoma were excluded. We also eliminated those without available blood test results for HBV infection. Finally, there were 652 patients confirmed to be diagnosed with GC qualified to be considered for the cases. The prevalence of HBV infection differed from time to time, and with the adoption of universal infant immunisation policy, the difference had become more apparent in recent years. Thus, we aimed to establish a control group matched with the GC group not only in age and sex, but also in the year of diagnosis. During the same period, there were 977 patients pathologically confirmed with nonmalignant diseases and with available blood test results for HBV infection, including 556 males and 421 females. All of them had received surgical treatment in our hospital, and the diagnoses were histopathologically confirmed to be nonmalignant diseases, including parotid adenolymphoma, benign meningioma, benign adrenal adenoma and so on. The list of pathologic diagnoses of the nonmalignant diseases is shown in Supplementary Table 1. None of these diseases had been reported to have any association with HBV infection. Patients with a recorded history of malignant diseases were excluded. However, there were not enough male patients for the controls to build a 1 : 1 case–control study if we were to include all 652 eligible patients in the cases, especially for the older patients (>60 years old). We randomly selected 580 patients from the qualified candidates for both the cases and controls to generate a 1 : 1 case–control study, with the balance of age, sex and year of diagnosis considered (Table 1). Baseline characteristics, blood test results for HBV infection and risk factors for GC were retrospectively collected from medical records. Family history of GC (no/yes), ABO blood type (A/B/AB/O), history of chronic gastritis (no/yes), smoking (never/past and/or current), alcohol consumption (never/past and/or current), diabetes mellitus (no/yes) and hepatitis C virus (HCV) infection (no/yes) were collected for both the cases and controls. Information on Hp infection (negative/positive) was available only for partial cases (42.6%, $n=247$). There was no information on Hp infection for the controls. In addition, history of chronic gastritis was only defined by medical history, and the results of gastroscopy and pathologic findings were not taken into consideration. This was because most controls did not receive a gastroscope inspection.

Serologic assay for HBV infection. For every patient in our hospital, a blood test for HBV infection was encompassed in routine examinations at the first visit. Blood samples were collected for the separation of serum samples that were used for the test of HBV infection. Several HBV-related antibodies and antigens were tested, including HBsAg, antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe) and antibody to hepatitis B core antigen (anti-HBc). Enzyme-linked immunosorbent assay was applied for this test. We had specific workers responsible for sending blood samples for tests in time to guarantee the quality of the test. Besides, qualified positive and negative control serum provided by the Ministry of Health of the People's Republic of China was used in this test to ensure the accuracy of the test.

Clinical significance of HBV-related antibodies and antigens. HBsAg: the serum maker for current HBV infection, including chronic hepatitis B and inactive HBsAg carrier state. Anti-HBs: a protective antibody in HBV infection. Anti-HBs positive indicates the acquisition of immune for HBV infection, and such a state may result from both current or prior HBV infection and hepatitis B vaccination. HBeAg: HBeAg positive indicates a high level of HBV DNA (200 000–2 billion IU ml^{-1}) in initial phase of HBV infection. However, in chronic hepatitis B, a portion of patients

Table 1. Characteristic of study population

Characteristic	Gastric cancer group ($n=580$) No. (%)	Control group ($n=580$) No. (%)	*P*-value
Age (years)			0.77
<50	144 (24.8)	134 (23.1)	
50–60	207 (35.7)	215 (37.1)	
>60	229 (39.5)	231 (39.8)	
Sex			0.61
Male	406 (70.0)	398 (68.6)	
Female	174 (30.0)	182 (31.4)	
Year of diagnosis			0.13
2007	80 (13.8)	80 (13.8)	
2008	236 (40.7)	204 (35.2)	
2009	264 (45.5)	296 (51.0)	

were found to be HBeAg negative with a lower level of HBV DNA (2000–20 million IU ml^{-1}). Anti-HBe: appearance of anti-HBe after clearance of HBeAg represents immune activation, and combined with HBsAg positive indicates inactive HBsAg carrier state. Anti-HBc: a serum marker for current or prior HBV infection. The clinical value of HBV-related antibodies and antigens were referenced to the American Association for the Study of Liver Diseases (AASLD) Practice Guideline: Chronic Hepatitis B: Update 2009.

Test for Hp infection. The rapid urease test was applied for the measurement of Hp infection. During gastroscopy, one biopsy specimen from the antrum and another from the corpus were obtained from each patient. The specimens were subjected to a rapid urease test to detect the presence of Hp urease. If the colour changed from yellow to pink within 24 h of incubation at room temperature, a positive result was reported. If the colour kept unchanged, the result was reported to be negative. In our retrospective collection, only 42.6% patients with GC had the test. According to the explanation of the staff members from the Department of Endoscopy, whether the test would be done or not depended on two main factors. One was the readiness of the patients, and the other was of the doctors. Some of them paid more attention to the biopsy for the diagnosis of GC whereas the test for Hp was ignored.

Statistical analysis. The statistical analyses were performed with SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). A two-tailed P-value of <0.05 was considered statistically significant. Differences in baseline parameters (age, sex and year of diagnosis) between the cases and controls were evaluated by χ^2 test. Binary logistic regression analysis was used to analyse the associations of GC and the characteristics of family history of GC, ABO blood type, history of chronic gastritis, smoking, alcohol consumption, diabetes mellitus, HCV infection, HBV antibodies and antigens. Variables significant in the univariate analyses and baseline characteristics including age, sex, and year of diagnosis were included for multivariate analyses. Multivariate logistic regression analysis was used to estimate the (AORs) and 95% confidence interval (CI).

RESULTS

Basic characteristics of the cases and controls. A total of 580 cases were matched to 580 controls. The ratio of case–control was 1 : 1. As for the distribution of age, patients were classified into three groups: <50 years old, 50–60 years old and >60 years old. The number of patients classified into the three groups were 144 (24.8%), 207 (35.7%), and 229 (39.5%) in the cases, and 134 (23.1%), 215 (37.1%) and 231 (39.8%) in the controls respectively. A male predominance was found in the cases (70.0%, $n=406$), and the matched controls had a similar distribution of sex with 398 (68.6%) male patients. The numbers of patients diagnosed in 2007, 2008 and 2009 were 80 (13.8%), 236 (40.7%) and 264 (45.5%) in the cases, and 80 (13.8%), 204 (35.2%) and 296 (51.0%) in the controls respectively. The cases and controls were well matched in the distribution of age, sex and year of diagnosis ($P=0.77$, $P=0.61$ and $P=0.13$ respectively, Table 1).

The general study population. There were 41 (7.1%) patients identified to have a family history of GC in the cases, whereas there were only 5 (0.9%) such patients in the controls. The numbers of patients with a history of chronic gastritis were 76 (13.1%) and 21 (3.6%) in the cases and controls, respectively. The univariable logistic regression analyses showed that family history of GC and history of chronic gastritis were significantly associated with GC ($P<0.001$ and $P<0.001$, respectively). As for some other factors identified to increase risk of GC by some previous investigations, including smoking, alcohol consumption and history of diabetes, we did not find them to be associated with GC in this study (Table 2).

With regard to the HBV antibodies and antigens, there were 100 (17.2%) HBsAg-positive patients in the cases, whereas the prevalence rate was significantly lower in the controls with 70 (12.1%) HBsAg-positive patients (OR (95% CI): 1.52 (1.09–2.11), Table 2). For anti-HBs-positive characteristic, there were 327 (56.4%) and 364 (62.8%) patients in the cases and controls, respectively (OR (95% CI): 0.77 (0.61–0.97), Table 2). However, no differences were identified in the rates of HBeAg ($P=0.42$), anti-HBe ($P=0.10$) and anti-HBc ($P=0.26$) in the univariate logistic regression analyses.

As suggested by the clinical value of anti-HBs, being anti-HBs positive might result from both HBV infection and hepatitis B vaccination. However, according to the literature, hepatitis B vaccination was adopted for newborns since 1992 in China, and therefore most of the patients included in our study presented with being anti-HBs positive resulting from current or prior HBV infection. In addition, being anti-HBs positive actually had different clinical significance in patients with current HBV infection (HBsAg positive) and without current HBV infection (HBsAg negative), which would be explained in detail in the discussion. Thus, to authentically evaluate the value of anti-HBs in GC, the research patients should not be analysed as a general population but should be classified into two subgroups. Subgroup 1: patients with current HBV infection (HBsAg positive). Subgroup 2: patients without current HBV infection (HBsAg negative), including patients with resolved hepatitis B or without confirmable history of HBV infection, and it should be clarified that this subgroup of patients is possibly exposed to HBV infection previously and might have occult HBV infection. As a result, there were 3 (3.0%) and 1 (1.4%) anti-HBs positive patients in the cases and controls, respectively, in patients with current HBV infection (HBsAg positive); the difference was not statistically significant by univariate logistic regression analysis (AOR (95% CI): 2.13 (0.22–20.95)). In patients without current HBV infection (HBsAg negative), being anti-HBs positive was identified in 324 (67.5%) and 363 (71.2%) patients in the cases and controls, respectively; the difference was not significant either (AOR (95% CI): 0.84 (0.64–1.10)). Thus, anti-HBs was found not to be associated with GC in this study (Table 2).

In the multivariate logistic regression analysis, the association of HBsAg with GC was not only adjusted by family history of GC (no/yes) and history of chronic gastritis (no/yes), which were found to be significantly associated with GC in the univariate logistic analysis, but also by age (as a continuous variable), sex (male/female) and year of diagnosis (2007/2008/2009), which were controlled artificially and kept to be potential confounding factors. Anti-HBs was not included for the reason explained above. After adjustment by confounding factors, the association between HBsAg and GC was still significant, with an estimated AOR (95% CI) of 1.49 (1.06–2.10) (Table 3).

Subgroup analyses of patients without a family history of GC. Patients with and without family history of GC represented different populations with distinct genetic background, with genetic factors playing more important roles in the former, indicating the importance of HBsAg that might differ in the two subgroups. Thus, further analyses were conducted stratified by family history of GC (no/yes). The HBsAg was identified not to be related with GC in the subgroup with family history of GC ($P=0.67$, OR (95% CI): 1.66 (0.17–16.38) in the univariate analysis). However, HBsAg was significantly related with GC in both univariate (OR (95% CI): 1.43 (1.02–2.01)) and multivariate analysis (AOR (95% CI): 1.49 (1.06–2.11)) in the subgroup without family history of GC (Supplementary Tables 2 and 3).

Table 2. Associations of gastric cancer risk factors, hepatitis B virus antibodies and antigens with gastric cancer risk: univariate logistic regression analyses

Variable	Gastric cancer group ($n=580$) No. (%)	Control group ($n=580$) No. (%)	OR	95% CI	*P*-value
Family history of gastric cancer (no/yes)	539/41 (92.9/7.1)	575/5 (99.1/0.9)	8.75	3.43–22.30	<0.001
ABO blood group (A/B/AB/O)					0.51
O	221 (39.0)	245 (42.2)	1	Reference	
A	166 (29.3)	148 (25.5)	1.24	0.93–1.66	0.14
B	142 (25.0)	146 (25.2)	1.07	0.80–1.45	0.62
AB	38 (6.7)	36 (6.2)	1.17	0.72–1.91	0.53
History of chronic gastritis (no/yes)	504/76 (86.9/13.1)	559/21 (96.4/3.6)	4.01	2.44–6.60	<0.001
Smoking (never/past and/or current)	365/211 (63.4/36.6)	359/222 (61.7/38.3)	0.93	0.74–1.18	0.56
Alcohol consumption (never/past and/or current)	477/99 (82.8/17.2)	487/93 (84.0/16.0)	1.09	0.80–1.48	0.60
History of diabetes mellitus (no/yes)	539/41 (92.9/7.1)	547/33 (94.3/5.7)	1.26	0.79–2.03	0.34
HCV (negative/positive)	538/2 (99.6/0.4)	539/3 (99.4/0.6)	0.67	0.11–4.01	0.66
HBV					
HBsAg (negative/positive)	480/100 (82.8/17.2)	510/70 (87.9/12.1)	1.52	1.09–2.11	0.01
Anti-HBs (negative/positive)	253/327 (43.6/56.4)	216/364 (37.2/62.8)	0.77	0.61–0.97	0.03
HBeAg (negative/positive)	578/2 (99.7/0.3)	576/4 (99.3/0.7)	0.50	0.09–2.73	0.42
Anti-HBe (negative/positive)	380/200 (65.5/34.5)	406/174 (70.0/30.0)	1.23	0.96–1.57	0.10
Anti-HBc (negative/positive)	337/243 (58.1/41.9)	356/224 (61.4/38.6)	1.15	0.91–1.45	0.26
Subgroup 1					
Anti-HBs negative and HBsAg positive	97 (97.0)	69 (98.6)	1	Reference	
Anti-HBs positive and HBsAg positive	3 (3.0)	1 (1.4)	2.13	0.22–20.95	0.52
Subgroup 2					
Anti-HBs negative and HBsAg negative	156 (32.5)	147 (28.8)	1	Reference	
Anti-HBs positive and HBsAg negative	324 (67.5)	363 (71.2)	0.84	0.64–1.10	0.21

Abbreviations: anti-HBc = antibody to hepatitis B core antigen; anti-HBe = antibody to hepatitis B e antigen; anti-HBs = antibody to hepatitis B surface antigen; CI = confidence interval; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; OR = odds ratio. Subgroup 1 indicated patients with current HBV infection, and subgroup 2 indicated patients without current HBV infection, but possibly exposed to HBV infection previously and still had occult HBV infection.

Table 3. Association between HBsAg and gastric cancer adjusted by age, sex, year of diagnosis and risk factors in multivariate logistic regression analyses

Variable	AOR	95% CI	*P*-value
HBsAg (negative/positive)	1.49	1.06–2.10	0.02
Family history of gastric cancer (no/yes)	8.61	3.36–22.08	<0.001
History of chronic gastritis (no/yes)	4.04	2.44–6.68	<0.001
Sex (female/male)	0.99	0.76–1.28	0.93
Age (as a continuous variable)	1.00	0.99–1.01	0.54
Year of diagnosis (2007/2008/2009)	0.93	0.78–1.10	0.39

Abbreviations: AOR = adjusted odds ratio; CI = confidence interval; HBsAg = hepatitis B surface antigen.

Subgroup analyses of HBsAg-negative patients. We subdivided HBsAg-negative patients into four subgroups according to the status of anti-HBc and anti-HBs. Univariate and multivariate logistic regression analyses were conducted. We found that compared with HBsAg-negative/anti-HBc-negative/anti-HBs-negative subgroup, HBsAg-negative/anti-HBc-positive/anti-HBs-negative subgroup had a trend of positive association with GC (AOR (95% CI): 1.67 (0.997–2.80)). Compared with HBsAg-negative/anti-HBc-positive/anti-HBs-negative subgroup, the other three subgroups were statistically or had a trend to be negatively associated with GC (AORs (95% CI): 0.62 (0.39–0.996), 0.47 (0.28–0.49), 0.60 (0.36–1.003)) (Tables 4 and 5). These associations became more apparent in HBsAg-negative patients without a family history of GC (Supplementary Table 4).

The synergetic effects of HBV infection and blood type A. By subdividing patients into four subgroups according to ABO blood type (A/non-A) and HBsAg (negative/positive), it was found that compared with blood type non-A/HBsAg-negative population, blood type A/HBsAg-positive population was significantly and positively associated with GC (AOR (95% CI): 2.41 (1.28–4.53)). We also subdivided the general population into another four subgroups according to ABO blood type (A/non-A) and anti-HBc (negative/positive). Similarly, compared with blood type non-A/anti-HBc-negative population, blood type A/anti-HBc-positive population was significantly and positively associated with GC (AOR (95% CI): 1.62 (1.09–2.42)) (Table 6).

DISCUSSION

This was the first case–control study to investigate the association between HBV infection and GC. Being HBsAg positive was identified unprecedented to be significantly and independently associated with GC, especially for patients without family history of GC. Occult HBV infection was also found to have some association

Table 4. The subgroup analyses for patients with HBsAg negative: univariate logistic regression analyses

Variable	Gastric cancer group ($n=480$) No. (%)	Control group ($n=510$) No. (%)	OR	95% CI	*P*-value
ABO blood group (A/B/AB/O)					0.59
O	189 (40.3)	214 (42.3)	1	Reference	
A	135 (28.8)	132 (26.1)	1.16	0.85–1.58	0.35
B	112 (23.9)	131 (25.9)	0.97	0.70–1.33	0.84
AB	33 (7.0)	29 (5.7)	1.29	0.75–2.20	9.35
History of chronic gastritis (no/yes)	414/66 (86.3/13.8)	491/19 (96.3/3.7)	4.12	2.43–6.98	<0.001
Family history of gastric cancer (no/yes)	451/29 (94.0/6.0)	506/4 (99.2/0.8)	8.13	2.84–23.32	<0.001
Smoking (never/past and/or current)	311/166 (65.2/34.8)	317/193 (62.2/37.8)	0.88	0.68–1.14	0.32
Alcohol consumption (never/past and/or current)	397/80 (83.2/16.8)	430/80 (84.3/15.7)	1.08	0.77–1.52	0.64
History of diabetes mellitus (no/yes)	444/36 (92.5/7.5)	483/27 (94.7/5.3)	1.45	0.87–2.43	0.16
HBsAg negative (A)					0.12
HBsAg negative and anti-HBc negative and anti-HBs negative	105 (21.9)	111 (21.8)	1	Reference	
HBsAg negative and anti-HBc negative and anti-HBs positive	231 (48.1)	241 (47.3)	1.01	0.73–1.40	0.93
HBsAg negative and anti-HBc positive and anti-HBs negative	51 (10.6)	36 (7.1)	1.50	0.91–2.48	0.12
HBsAg negative and anti-HBc positive and anti-HBs positive	93 (19.4)	122 (23.9)	0.81	0.55–1.18	0.27
HBsAg negative (B)					0.12
HBsAg negative and anti-HBc positive and anti-HBs negative	51 (10.6)	36 (7.1)	1	Reference	
HBsAg negative and anti-HBc negative and anti-HBs positive	231 (48.1)	241 (47.3)	0.68	0.43–1.08	0.10
HBsAg negative and anti-HBc positive and anti-HBs positive	93 (19.4)	122 (23.9)	0.54	0.33–0.89	0.02
HBsAg negative and anti-HBc negative and anti-HBs negative	105 (21.9)	111 (21.8)	0.67	0.40–1.10	0.12

Abbreviations: anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen; CI = confidence interval; HBsAg = hepatitis B surface antigen; OR = odds ratio.

with GC. In addition, some synergistic effects between HBV infection and blood type A were identified.

Previous exploration has identified HBsAg as an independent risk factor for both liver cirrhosis and hepatocellular carcinoma (Lee *et al*, 2013a). Besides, liver cirrhosis has been reported to be a risk factor for gastric ulcer and GC (Zullo *et al*, 2003; Kirchner *et al*, 2011). Thus, the association of HBsAg with risk of GC may be linked by liver cirrhosis. It is possible that liver cirrhosis and/or HBV infection itself was responsible for increased risk of GC. On the other hand, HBV infection has been confirmed to be a risk factor for several cancers of other organs and tissues involved in HBV infection. Hepatocellular carcinoma is well known as one of the HBV-related cancers. As HBV infection also exists in gastric mucosa epithelial cells, it may be possible that HBV infection increases the risk of GC in a similar mechanism of HBV-related hepatocellular carcinoma. The HBV infection has been commonly recognised as a risk factor for hepatocellular carcinoma (Yu *et al*, 1990; Chen *et al*, 2006; Lee *et al*, 2013b). The mechanism of HBV-induced hepatocellular carcinoma has been thoroughly researched, and it is complex, including direct enhancement of chromosomal instability by integration of HBV DNA into the host genome that results in alterations of host gene expression and signalling pathways (Tan, 2011). Indirect mechanisms, such as persistent inflammation, oxidative stress, hypoxia caused by cirrhosis and sequential angiogenesis, are also raised up (Arzumanyan *et al*, 2013). Recently, the epigenetic changes generated by the HBV-encoded X (HBx) protein became another focus in the exploration for mechanism of HBV-induced hepatocellular carcinoma (Tan, 2011; Tian *et al*, 2013). However, to understand the authentic and detailed mechanism of HBV infection-related GC, more studies should be conducted.

The simultaneous seropositivity of HBsAg and anti-HBs (which meant being anti-HBs positive in HBsAg-positive patients) was not common in HBV infection that was reported to be <10% in the general population with HBV infection (Zhang *et al*, 2007; Liu *et al*, 2012; Seo *et al*, 2014). In our study, the rates of simultaneous seropositivity of HBsAg and anti-HBs in HBV infection patients were 3.0% and 1.4% in the cases and controls, respectively. Many studies have proved that patients with coexisting serological HBsAg and anti-HBs have more frequent mutations and variations in the antigenic epitopes of HBsAg that result in possible difficulty of virus clearance (Lada *et al*, 2006; Chen *et al*, 2011; Liu *et al*, 2012). In HBsAg-negative patients, anti-HBs was recognised as an antibody protecting from HBV infection. However, anti-HBs positive exhibited distinct clinical value in the subgroup with and without current HBV infection. Thus, to explore the value of anti-HBs in GC, patients should be categorised into two subgroups. In Table 2, the univariated analyses showed that anti-HBs was related with GC when patients were analysed as a whole, but no relationship was found when patients were stratified into two subgroups. This could be explained as follows. Because the coexistence of HBsAg and anti-HBs was rare, and HBsAg was positively associated with GC, it was predictable that the cases had a lower rate of being anti-HBs positive. Thus, the negative association between anti-HBs and GC in the analysis of general patients actually reflected the positive association between HBsAg and GC.

Anti-HBc positive (combined with anti-HBs positive or not) was found to be associated with increased risks of liver and pancreatic cancer compared with those never exposed to HBV infection (Brechot *et al*, 2001; Hassan *et al*, 2008). Occult HBV infection, which indicated the presence of HBV DNA in the serum

or liver in people lacking HBsAg in the serum outside the acute window phase period, was recurrently identified in anti-HBc-positive patients (Allain, 2004). However, in HBsAg-negative patients with liver cancer, not only the HBsAg-negative/anti-HBc-positive/anti-HBs-negative and HBsAg-negative/anti-HBc-positive/anti-HBs-positive patients, but also HBsAg-negative/HBcAb-negative/HBsAb-negative patients were found to have HBV DNA detected (Paterlini *et al*, 1990). Being anti-HBc positive (with or without anti-HBe positive) without being anti-HBs positive in HBsAg-negative patients was usually called anti-HBc only. Anti-HBc only was frequently found to be associated with HBV infection in blood transfusion, although the possibility of HBV infection in HBsAg-negative/anti-HBc-positive/anti-HBs-positive blood transfusion was lower (Mosley *et al*, 1995; Allain, 2004; Allain *et al*, 2013). It could be speculated that the rates of occult HBV infection in anti-HBc-only patients, HBsAg-negative/anti-HBc-positive/anti-HBs-positive patients and HBsAg-negative/anti-HBc-negative/anti-HBs-negative patients might be sequentially decreasing. These findings helped to understand the results by the analyses for HBsAg-negative subgroup in our study.

Table 5. The subgroup analyses for patients with HBsAg negative: multivariate logistic regression analyses

Variable	AOR	95% CI	*P*-value
HBsAg negative (A)			0.04
HBsAg negative and anti-HBc negative and anti-HBs negative	1	Reference	
HBsAg negative and anti-HBc negative and anti-HBs positive	1.04	0.74–1.45	0.83
HBsAg negative and anti-HBc positive and anti-HBs negative	1.67	0.997–2.80	0.051
HBsAg negative and anti-HBc positive and anti-HBs positive	0.79	0.533–1.17	0.23
HBsAg negative (B)			0.04
HBsAg negative and anti-HBc positive and anti-HBs negative	1	Reference	
HBsAg negative and anti-HBc negative and anti-HBs positive	0.62	0.39–0.996	0.048
HBsAg negative and anti-HBc positive and anti-HBs positive	0.47	0.28–0.49	0.004
HBsAg negative and anti-HBc negative and anti-HBs negative	0.60	0.36–1.003	0.051

Abbreviations: anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen; CI = confidence interval; HBsAg = hepatitis B surface antigen; OR = odds ratio. The results were adjusted by history of chronic gastritis (no/yes), family history of gastric cancer (no/yes), age (as a continuous variable), sex (male/female) and year of diagnosis (2007/2008/2009).

Blood type A was found to be associated with a higher risk of GC (Wang *et al*, 2012). It was also found to synergistically increase the risk of pancreatic cancer with HBV infection (Wang *et al*, 2012). In our study, although the univariate logistic regression analysis found no association of ABO blood group with GC, multivariate logistic regression analysis showed that blood type A had a trend to be positively associated with GC compared with blood type O after adjustment by risk and potential confounding factors (AOR (95% CI): 1.30 (0.97–1.74), $P = 0.08$, Supplementary Table 5). Moreover, we found that blood type A also had some synergistic effects with HBV infection in GC (Table 6). Additional blood type A increased the risk for GC in HBsAg-positive patients, with AOR increasing from 1.49 to 2.41. Such a synergism was also found in pancreatic cancer (Wang *et al*, 2012) and extrahepatic cholangiocarcinoma (Zhou *et al*, 2013). However, the mechanism behind that has not been illuminated. Kai *et al* (2012) found that HCV infection was associated with GC in patients with liver cancer. There were some limitations in that study such as they did not include possible occult HBV coinfection as well as liver cirrhosis in the analyses. It was reported that the incidence of occult HBV infection was high in patients with HCV infection, and might increase the risk of liver cancer (Cardoso *et al*, 2013; Squadrito *et al*, 2013). In our study, the incidence of HCV was too low to identify any difference, and to investigate the aetiologic effect of HCV in GC in China, a larger case–control study or prospective study would be more convincing.

This was a well-matched case–control study, but some limitations should not be neglected. The most important one was the lack of information about Hp infection in the controls, and

Table 6. Univariate and multivariate logistic regression analyses for the synergistic effect of blood type A and HBV infection in gastric cancer

	Univariate analyses			Multivariate analyses		
Variable	**OR**	**95% CI**	***P*-value**	**AOR**	**95% CI**	***P*-value**
ABO blood group and HBsAg			0.04			0.03
Blood type non-A and HBsAg negative	1	Reference		1	Reference	
Blood type A and HBsAg negative	1.15	0.86–1.52	0.35	1.20	0.90–1.60	0.22
Blood type non-A and HBsAg positive	1.42	0.96–2.09	0.08	1.35	0.90–2.03	0.15
Blood type A and HBsAg positive	2.17	1.17–4.04	0.02	2.41	1.28–4.53	0.01
ABO blood group and anti-HBc			0.26			0.13
Blood type non-A and anti-HBc negative	1	Reference		1	Reference	
Blood type A and anti-HBc negative	1.09	0.78–1.53	0.60	1.12	0.79–1.58	0.54
Blood type non-A and anti-HBc positive	1.10	0.83–1.45	0.50	1.07	0.80–1.42	0.66
Blood type A and anti-HBc positive	1.49	1.01–2.20	0.046	1.62	1.09–2.42	0.02

Abbreviations: anti-HBc = antibody to hepatitis B core antigen; AOR = adjusted odds ratio; CI = confidence interval; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; OR = odds ratio. There were 567 and 575 cases included in the analyses in gastric cancer and control group respectively after cases without information for ABO blood group being excluded. The multivariate logistic regression analyses were adjusted by history of chronic gastritis (no/yes), family history of gastric cancer (no/yes), age (as a continuous variable), sex (male/female) and year of diagnosis (2007/2008/2009).

thus the associations of HBV and GC were not adjusted by Hp infection. To make up for this flaw, we analysed the association of anti-HBs (positive/negative) and HBsAg (positive/negative) with Hp (positive/negative). No association was found (Supplementary Table 6). In addition, some previous studies also suggested Hp infection was not related with HBV infection in gastric mucosa. Chen *et al* (2004) reported that there was no difference in the expression of HBV antigens in the Hp-positive and -negative gastric antrum mucosa in patients with HBV infection. Another study by Kirchner *et al* (2011) revealed that the association of Hp infection with gastric ulcers was weak in liver cirrhosis patients, suggesting the existence of other important aetiologic factors for ulcers in this population. From the above studies, although we did not include Hp infection in logistic regression analyses, some proofs supported the lack of interaction between HBV and Hp infection. More qualified case–control studies with the information of Hp infection status included are needed in future. In addition, we failed to choose healthy people as the controls, and the heterogeneity of patients with various kinds of benign diseases might have some influences in our study. In addition, we could not analyse the role of liver cirrhosis and changes of liver function as well as subsequent changes in life style in GC owning to lack of relevant data in our study. Moreover, this study was retrospectively conducted, and thus its efficacy to test a risk factor in the aspect of direct causal relationship was relatively weak. The association of HBV infection with the risk of GC needs to be confirmed in future prospective study.

In conclusion, this case–control study is the first one to discover the association between HBV infection and GC. Gastric cancer was found to be associated with a significantly higher rate of positive HBsAg, indicating HBV infection may be a possible risk factor for GC. Occult HBV infection and synergistic effects of HBV infection with blood type A were also found to have some roles in the risk of GC. Future studies need to verify the existence of HBV DNA and antigens in GC, and large-scale prospective investigations are warranted to testify the conclusions, and the mechanisms need to be more specifically and thoroughly investigated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

In the initial phase of this study, RHX, XLW and YHL designed the study. XLW, MZQ, YXH and RYW participated in the clinical data collection of both cases and controls. DSW, FW and HYL participated in the collection of information on hepatitis B virus infection and *Helicobacter pylori* infection. YJ, WWC and DSZ performed the statistical analysis. XLW drafted the manuscript. MZQ, YJ and FHW revised the manuscript. RHX coordinated work and helped to draft and revise the manuscript. All authors read and approved the final manuscript.

After we received the request for revision of our manuscript, XLW, YJ and DSZ participated in the collection of supplementary information needed to revise the manuscript and answer the questions of reviewers, such as the diagnoses of non-malignant diseases, the information of liver function and liver cirrhosis. XLW and DSZ wrote the rebuttal letter and revised the manuscript. RHX helped to revise the manuscript. The revised manuscript was read and approved by all authors.

Explanation for the designation of co-first authors: In the initial phase of this study, XLW, MZQ and YJ had main contributions, so they were designed as co-first authors when we submitted the manuscript. However, in the revision phase of the study, the contribution of DSZ made us reconsider his author order. All the authors agreed that DSZ should also be listed as a co-first author.

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