Research Article

Hepatitis B Virus Infection and Risk of Nasopharyngeal Carcinoma in Southern China

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Abstract

Background: Whether or not hepatitis B virus (HBV) infection plays a role in the development of nasopharyngeal carcinoma (NPC) is largely unknown. Our study aimed to assess the association between HBV infection and the risk of NPC in Southern China.

Methods: We conducted a case–control study including 711 NPC cases and two groups of controls. The first control group consisted of 656 individuals with other benign tumors unrelated to HBV infection and the second group consisted of 680 healthy population controls. Multivariable ORs and corresponding 95% confidence intervals (CI) for NPC were estimated by logistic regression.

Results: Patients with NPC had higher prevalence of antibodies against hepatitis B core antigen–positive [anti-HBc (+)] 47.26% compared with either benign tumor controls (39.33%; P < 0.01) or healthy controls (41.18%; P = 0.04). In multivariable models adjusting for a set of risk factors for NPC, anti-HBc (+) was significantly associated with a higher risk of NPC [adjusted OR (AOR), 1.40; 95% CI, 1.12–1.74 compared with the benign tumor controls and AOR, 1.48; 95% CI, 1.05–2.08 compared with the healthy controls]. The association was not modified by hepatitis B surface antigen (HBsAg) status. Finally, compared with the healthy controls, individuals with both anti-HBc (+) and HBV antibodies had largely increased risk of NPC [AOR, 141.82; 95% CI, 68.73–292.62].

Conclusion: Our study suggests that HBV infection is associated with NPC risk in Southern China.

Impact: Prevention for HBV infection may play a role in the development of NPC. Cancer Epidemiol Biomarkers Prev; 24(11); 1766–73. ©2015 AACR.

Introduction

Nasopharyngeal carcinoma (NPC) is a relatively rare epithelial malignancy in North America and other Western countries (world age-standardized incidence rate: <1 per 100,000 person-years), whereas in contrast, it is endemic in Southern China (world age-standardized incidence rate: 20–50 per 100,000 person-years among males; refs. 1–6). Multiple factors, including Epstein–Barr virus (EBV) infection, environmental exposure, and genetics, are likely interplaying with each other closely in the development of NPC (7). EBV infection is considered a necessary etiologic factor for NPC to date. Reactivation of EBV has been observed in patients with NPC several years before disease onset (8, 9), and almost all undifferentiated forms of NPC cells harbor EBV DNA (10). Multiple EBV genes, latent and lytic in nature, have been shown to contribute to the malignant transformation of premalignant nasopharyngeal epithelial cells (11, 12). However, the roles of other viral infections in the development of NPC have rarely been explored.

Hepatitis B virus (HBV) is a hepatotropic and lymphotrophic virus that can infect and replicate in lymphocytes and peripheral blood mononuclear cells (13). HBV infection may increase the risk of hepatocellular carcinoma (14), cholangiocarcinoma (15), non–Hodgkin lymphoma (16), and pancreatic cancer (17). Host immune responses toward persistent HBV infection may promote chronic inflammation and cell proliferation and subsequently induce oncogenic transformation of the infected cells (18–20). NPC tumor cells are known to be heavily infiltrated by lymphocytes (21). B lymphocytes can be infected by EBV and HBV simultaneously (22–24). The proliferated B lymphocytes induced by HBV infection may in succession activate the latent EBV and trigger EBV shedding in nasopharynx, leading to EBV infection in the nasopharyngeal epithelial cells (25). Moreover, inflammatory cytokines may induce gene mutation of the epithelial cells and further facilitate EBV infection (26, 27).

Around 350 million people are estimated to be chronically infected with HBV worldwide, among whom approximately 75%...
live in the Asia Pacific region, mostly Southern China (28, 29). From 1992, the Ministry of Health in China had recommended hepatitis B vaccination as a part of the routine infant immunization schedule with however no subsidy. From 2002 onward, subsidy for this vaccination was provided to cover all infants and has thereafter largely reduced HBV infection rate among Chinese children (30, 31). According to the survey conducted by the Disease Control and Prevention Center of China in 2006, the prevalence of hepatitis B surface antigen (HBsAg) and antibodies against hepatitis B core antigen (anti-HBc) was 1.00% and 4.10% in children of 1 to 4 years; the respective prevalence among children in Guangdong province was 1.92% and 3.03% (29, 30). On the other hand, HBV infection remains highly prevalent in the population over 5 years of age; among individuals at the age of 5 to 59 years, the prevalence of HBsAg was 8.75% and of anti-HBc 41.61%. The prevalence was even higher in Guangdong with a prevalence of HBsAg as 13.19% and of anti-HBc as 44.43% (29, 30).

In China, a total of eight provinces had a prevalence of HBsAg above 10%, including Guangdong, Guangxi, Hainan, Zhejiang, Fujian, Jiangxi, Hubei, and Sichuan. These areas appear to largely coincide with the endemic areas of NPC incidence in China. Among the six provinces with the high incidence rates of NPC (Guangdong, Guangxi, Hainan, Fujian, Jiangxi, and Hunan), five (Guangdong, Guangxi, Hainan, Fujian, and Jiangxi) had also >10% prevalence of HBsAg positiveness (32, 33).

To this end, we hypothesized that HBV infection might contribute to a higher risk of NPC in Southern China. We conducted a case–control study in Guangdong Province to test this hypothesis and to further assess whether HBV may interacts with EBV in promoting the development of NPC.

Materials and Methods

Study population

The present study used two groups of controls: the first including individuals with other benign tumors and the second including healthy population controls. Patients with NPC and benign tumor controls were both newly diagnosed and treated in Sun Yat-sen University Cancer Center (Guangzhou, Guangdong Province, China) from January 1, 2008, to May 30, 2013. Benign tumor controls were patients with other benign tumors that are believed to be unrelated to HBV infection, including polyp of vocal cord gall, benign adrenal tumors, ovarian cyst, renal cyst, polycystic kidney, benign neoplasm of thyroid gland, uterine fibroids, hyperplasia of the cervical squamous epithelium, bladder polyps, breast fibroadenoma, hemangioma, and benign brain tumor. NPC and other benign tumors were all histologically confirmed. A total of 661 eligible benign tumor controls were consecutively identified during the study period. To balance the recruitment times between cases and controls, a total of 722 cases, including 568 Cantonese cases, were randomly selected from the total of 6,963 patients with NPC identified during the same period. Cases and controls were frequency-matched at a ratio of 1 to 1.1 and by age (in 5-year groups) and sex. We further excluded 11 NPC cases with known infection of hepatitis A virus (HAV), HCV, hepatitis delta virus (HDV), HEV, and HIV. Similarly, 5 controls with known infection of HCV or HEV were excluded, leaving 656 controls in the analysis. The pathologic diagnosis was World Health Organization (WHO) type III for 689 cases (95.92%), type II for 21 cases (2.95%), and type I for one case (0.14%). All relevant information, including sex, age, place of birth, cigarette smoking, alcohol drinking, family history of cancer, hepatitis B, and liver function, was collected through detailed medical records reviews for both cases and controls. In addition, information on immunoglobulin A antibodies against EBV capsid antigen (VCA-IgA) and early antigen (EA-IgA) titers was collected for the cases.

The healthy population controls were selected from individuals that participated in a population-based NPC screening program in Sihui county, Guangdong Province, China (34). In total, 5481 eligible individuals at the age of 30 to 59 years in Sihui were recruited in 2008 to the screening program. Blood samples were collected at recruitment and tested for VCA-IgA and EA-IgA using immunofluorescence assay (IFA) method for all participants. The high-risk individuals (i.e., VCA-IgA ≥ 1:40 or both VCA-IgA and EA-IgA ≥ 1:10) were referred to nasopharyngeal endoscopy examination and/or pathologic biopsy. All participants also completed an in-person interview collecting information on demographics as well as risk factors for NPC. During the first year of follow-up after recruitment, a total of eight individuals were identified as incident NPC cases, leaving 5,373 as non-NPC healthy participants. A total of 683 individuals were randomly selected from these participants and were frequency matched to the Cantonese NPC cases of the present study (n = 568) at a ratio of 1 to 1.2 and by age (in two groups: ≤40 or >40 years) and sex. Among these controls, 3 individuals with infection of HAV or HCV were identified and excluded, leaving 680 healthy controls in the analysis.

Serologic assays

For every patient with a newly diagnosed tumor in Sun Yat-sen University Cancer Center during the study period, 6-mL fasting blood sample was routinely collected before treatment and sent to the clinical laboratories for testing of liver function, as well as the infections of HAV, HBV, HCV, HDV, HEV, and HIV. As a result, for both the NPC cases and benign tumor controls, an ELISA (Kehua Bio-Engineering, Co., Ltd.) was used to test the serum samples for HBsAg, antibodies against HBsAg (anti-HBs), hepatitis B e antigen (HBcAg), antibodies against (anti-HBc), anti-HBE, HAV, HCV, HDV, HEV, and HIV. The serum samples were also used to test the concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as the comparability of ALT and AST concentration, 82 subjects who were selected from these participants and were frequency matched to the Cantonese NPC cases of the present study and were referred to nasopharyngeal endoscopy examination and/or pathologic biopsy. All participants also completed an in-person interview collecting information on demographics as well as risk factors for NPC. During the first year of follow-up after recruitment, a total of eight individuals were identified as incident NPC cases, leaving 5,373 as non-NPC healthy participants. A total of 683 individuals were randomly selected from these participants and were frequency matched to the Cantonese NPC cases of the present study (n = 568) at a ratio of 1 to 1.2 and by age (in two groups: ≤40 or >40 years) and sex. Among these controls, 3 individuals with infection of HAV or HCV were identified and excluded, leaving 680 healthy controls in the analysis.
were required to be correctly classified as indicated in each kit. An inconsistent result for a QC sample prompted a repeated testing. All of the proportions of agreement for positive and negative QC samples of HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc were 100% [95% confidence interval (CI), 1.00 to 1.00] in the present study. In addition, routine external quality assessment with the pooled serum provided by the Ministry of Health of the PR China was conducted in every day. The intraclass correlation coefficients (ICC) for the pooled QC samples of HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc were excellent (>0.9). The coefficients of variation (CV) for the pooled QC samples of HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc were all less than 20% (Supplementary Table S1). Only positive QC samples were used for ALT and AST assays. The ICCs of the QC samples for ALT and AST were 0.999 (95%CI, 0.999–1.000) and 1.000 (95% CI, 1.000–1.000), and the normal ranges were defined as 0–40 and 0–45 international units per liter by the kits. The CVs of the QC samples for ALT and AST were 2.294% (95%CI, 2.072–2.569) and 2.023% (95%CI, 1.829–2.265) in this study, within the range (20%) of allowed total error as indicated by the kits. EBV VCA-IgA and EA-IgA were measured by in-house IFA for both the cases and healthy population controls as previously described (34), and a positive result was defined if the titer was ≥1:10. A pooled serologic sample as control sample for EBV VCA-IgA and EA-IgA testing has been used in the Sun Yat-sen University Cancer Center since 1980s, and the CVs of these two measurements for the pooled samples were 8.04% and 8.13%. Among the healthy controls, as our previous study reported (34), 5% of the stored serum samples were randomly selected for retesting. The ICCs of the EBV VCA-IgA and EA-IgA were 0.740 (95% CI, 0.515–0.870) and 0.420 (95% CI, 0.067–0.680).

Definition for HBV infection

HBV infection status was divided into three categories according to the status of HBsAg and anti-HBc. Individuals with both HBsAg negative and anti-HBc negative, that is, HBsAg (−)/anti-HBc (−), were defined as never infected with HBV; those with HBsAg (+)/anti-HBc (+) were defined as chronically infected with HBV; and those with HBsAg (−)/anti-HBc(+) were defined as previously infected with HBV (17).

Statistical analysis

We used a χ2 test to compare the differences of baseline characteristics between cases and controls. Unconditional logistic regression was applied to calculate the ORs and their corresponding 95% CIs. Because of the collinearity among the tested HBV markers and the HBV infection categories, we included the five HBV markers and the three HBV infection categories individually into the multivariable logistic regression to assess the AORs after adjusting for age (as a continuous variable), sex, ALT/AST concentrations (i.e., normal or abnormal), alcohol drinking, cigarette smoking, family history of cancer, or EBV serologic antibodies (i.e., any of VCA-IgA and EA-IgA positive or both of VCA-IgA and EA-IgA negative). To assess the AORs for ALT/AST concentrations, alcohol drinking, cigarette smoking, and family history of cancer, these variables were mutually adjusted for each other, in addition to age, sex, and HBV infection categories. Second, to assess the potential modification effect of other risk factors on the association between HBV infection and NPC, we performed stratified analyses by ALT concentration, smoking, family history of cancer, and EBV infection status respectively. Potential interactions between HBV infection and these risk factors were assessed using likelihood ratio test. Finally, as some of our study participants might have received a vaccination for HBV and to alleviate the concern regarding the unknown impact of HBV vaccination on the studied associations, in a sensitivity analysis, we repeated the analysis after excluding individuals with anti-HBs (+)/anti-HBc (−).

SAS software (Version 9.2, SAS Institute Inc.) was used for data analysis. The P value was based on two-sided tests and <0.05 was set as the criterion for statistical significance. This study was approved by the Institutional Research Ethics Committee of Sun Yat-sen University Cancer Center (Z2014-02001). Written informed consent was obtained from all participants.

Results

The baseline characteristics for NPC cases and the 2 groups of controls are presented in Table 1. The mean age (±SD) was 43.16 (±11.86) years for NPC cases, 43.61 (±12.05) years for benign tumor controls, and 43.79 (±7.48) years for healthy controls. Most of the NPC cases and controls were female (P = 0.68).

Compared with benign tumor controls, patients with NPC had significantly higher percentage of anti-HBc (+) (47.26% vs. 39.33%, P < 0.01; Table 2). In multivariable models, individuals with anti-HBc (+) were also more likely to be diagnosed with NPC (AOR, 1.40; 95% CI, 1.12–1.74) and the association was not obviously modified by HBsAg status; AOR 1.40 (95% CI,
1.00–1.94) for chronic infection [HBsAg(+)/anti-HBc(−)] and AOR 1.40 (95% CI, 1.09–1.79) for previous infection [HBsAg(−)/anti-HBc(−)]. Similarly, when comparing NPC cases with healthy population controls, anti-HBc(−) was also associated with a higher risk of NPC (AOR, 1.48; 95% CI, 1.05–2.08). The association appears similar for chronic and previous HBV infection (Table 2). However, abnormal ALT concentration was more frequent among individuals with chronic infection (22.1%, 43 of 195) than among individuals with previous infection (9.52%, 38 of 399; P < 0.01).

After adjusting for age, sex, and HBV infection categories as well as mutually adjusting for each other, a family history of cancer (AOR, 3.88; 95% CI, 2.73–5.53), an abnormal ALT concentration (AOR, 1.62; 95% CI, 1.07–2.44), and smoking (AOR, 1.54; 95% CI, 1.06–2.22) were all associated with a higher risk of NPC when comparing patients with NPC with the benign tumor controls (Table 3). Individuals with abnormal ALT concentration or a family history of cancer had also an increased risk of NPC (AOR, 1.98; 95% CI, 1.03–3.83 and AOR, 2.05; 95% CI, 1.28–3.28), when comparing patients with NPC with the healthy controls. A very strong association between EBV infection and NPC was observed (AOR, 78.22; 95% CI, 49.65–123.23) as well. However, cigarette smoking appeared to have no relationship with the risk of NPC in this comparison (Table 3).

There was no statistically significant interaction between HBV infection and ALT concentration, cigarette smoking, family

### Table 2. The associations of HBV infection with nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (Cantonese case) [N = 711 (568)]</th>
<th>Benign tumor controls (N = 656)</th>
<th>Healthy population controls (N = 680)</th>
<th>All cases and benign tumor controls, multivariablea</th>
<th>Cantonese cases and healthy population controls, multivariableb</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg(+)</td>
<td>599 (474)</td>
<td>84.25 (83.45)</td>
<td>573 (87.35)</td>
<td>583 (85.74)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>HBsAg(+)</td>
<td>112 (94)</td>
<td>15.75 (16.55)</td>
<td>83 (12.65)</td>
<td>97 (14.26)</td>
<td>1.25 (0.91–1.71)</td>
</tr>
<tr>
<td>Anti-HBs(−)</td>
<td>241 (192)</td>
<td>33.90 (33.80)</td>
<td>231 (35.21)</td>
<td>238 (35.00)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Anti-HBc(−)</td>
<td>470 (376)</td>
<td>66.10 (66.20)</td>
<td>425 (64.79)</td>
<td>442 (65.00)</td>
<td>1.06 (0.84–1.34)</td>
</tr>
<tr>
<td>HBeAg(+)</td>
<td>693</td>
<td>97.47</td>
<td>638 (97.26)</td>
<td>1 (ref.)</td>
<td></td>
</tr>
<tr>
<td>HBeAg(+)</td>
<td>18</td>
<td>2.53</td>
<td>18 (2.74)</td>
<td>0.82 (0.40–1.65)</td>
<td>0.58 – –</td>
</tr>
<tr>
<td>Anti-HBe(−)</td>
<td>473</td>
<td>66.53</td>
<td>459 (69.97)</td>
<td>1 (ref.)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBe(−)</td>
<td>238</td>
<td>33.47</td>
<td>197 (30.03)</td>
<td>1.17 (0.93–1.48)</td>
<td>0.19 – –</td>
</tr>
<tr>
<td>Anti-HBc(−)</td>
<td>375 (290)</td>
<td>52.74 (51.06)</td>
<td>398 (60.67)</td>
<td>400 (58.82)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Ever as smoking</td>
<td>224 (184)</td>
<td>31.50 (32.39)</td>
<td>175 (26.68)</td>
<td>1.40 (1.02–1.74)</td>
<td>0.01 1.48 (1.05–2.08)</td>
</tr>
<tr>
<td>Never as smoking</td>
<td>356 (278)</td>
<td>47.26 (48.94)</td>
<td>258 (39.32)</td>
<td>280 (41.18)</td>
<td>1 (ref.)</td>
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<tr>
<td>ABV infection status</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg(−)/anti-HBc(−)</td>
<td>375 (290)</td>
<td>52.74 (51.06)</td>
<td>398 (60.67)</td>
<td>400 (58.82)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Ever as smoking</td>
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<td>31.50 (32.39)</td>
<td>175 (26.68)</td>
<td>1.40 (1.02–1.74)</td>
<td>0.01 1.48 (1.05–2.08)</td>
</tr>
</tbody>
</table>

aAORs were adjusted for age, sex (as a continuous variable), alcohol drinking, cigarette smoking, ALT concentration, AST concentration, and family history of cancer in all cases and benign tumor controls.

### Table 3. The associations of liver function, cigarette smoking, alcohol drinking, family history of cancer, and EBV antibodies with the risk of NPC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (Cantonese case) [N = 711 (568)]</th>
<th>Benign tumor controls (N = 656)</th>
<th>Healthy population controls (N = 680)</th>
<th>All cases and benign tumor controls, multivariablea</th>
<th>Cantonese cases and healthy population controls, multivariableb</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>615 (491)</td>
<td>87.48 (87.52)</td>
<td>596 (91.30)</td>
<td>635 (93.52)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>88 (70)</td>
<td>12.52 (12.48)</td>
<td>57 (87.40)</td>
<td>44 (6.48)</td>
<td>1.62 (1.07–2.44)</td>
</tr>
<tr>
<td>AST concentration</td>
<td>703 (561)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>684 (555)</td>
<td>97.30 (98.93)</td>
<td>639 (97.56)</td>
<td>658 (96.91)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>19 (6)</td>
<td>2.70 (1.07)</td>
<td>16 (2.44)</td>
<td>21 (3.09)</td>
<td>0.76 (0.36–1.64)</td>
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<td>Cigarette smoking</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever as smoking</td>
<td>989 (869)</td>
<td>82.84 (82.57)</td>
<td>563 (85.82)</td>
<td>538 (79.12)</td>
<td>1 (ref.)</td>
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<tr>
<td>Alcohol drinking</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Never as smoking</td>
<td>711 (681)</td>
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<tr>
<td>Ever as smoking</td>
<td>696 (557)</td>
<td>97.89 (98.06)</td>
<td>637 (97.10)</td>
<td>655 (96.32)</td>
<td>1 (ref.)</td>
</tr>
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<td>Family history of cancer</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>555 (438)</td>
<td>78.06 (77.11)</td>
<td>610 (92.99)</td>
<td>611 (89.85)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Yes</td>
<td>150 (130)</td>
<td>21.94 (22.89)</td>
<td>46 (7.01)</td>
<td>69 (10.15)</td>
<td>3.88 (2.73–5.53)</td>
</tr>
<tr>
<td>EBV antibodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCA-IgA and EA-IgA(+)</td>
<td>32 (24)</td>
<td>4.61 (4.33)</td>
<td>–</td>
<td>–</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>VCA-IgA or EA-IgA(−)</td>
<td>662 (530)</td>
<td>95.39 (95.67)</td>
<td>532 (78.24)</td>
<td>–</td>
<td>1 (ref.)</td>
</tr>
</tbody>
</table>

aAORs were adjusted for sex, age (as a continuous variable), HBV infection status, alcohol drinking, cigarette smoking, ALT concentration, AST concentration, and family history of cancer in all cases and benign tumor controls.

bAORs were adjusted for sex, age (as a continuous variable), HBV infection status, alcohol drinking, cigarette smoking, ALT concentration, AST concentration, family history of cancer, and EBV antibodies in the cases of Guangdong province population and healthy population controls.
Ye et al.

Table 4. The modification effects by liver function, smoking, family history of cancer, and EBV infection between the association of anti-HBc and the risk of NPC with stratified analyses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (Cantonese cases)</th>
<th>Benign tumor controls (N = 655)</th>
<th>Healthy population controls (N = 680)</th>
<th>All cases and benign tumor controls multivariable*</th>
<th>Cantonese cases and healthy population controls multivariableb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>AORs (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Anti-HBc + ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/normal ALT</td>
<td>372 (255)</td>
<td>655</td>
<td>679</td>
<td>1 (ref.)</td>
<td>0.85</td>
</tr>
<tr>
<td>Anti-HBc(−)/abnormal ALT</td>
<td>37 (28)</td>
<td>27 (412)</td>
<td>1.65 (0.94–2.87)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/normal ALT</td>
<td>283 (234)</td>
<td>227 (34.66)</td>
<td>1.41 (112–178)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/abnormal ALT</td>
<td>51 (42)</td>
<td>16 (2.36)</td>
<td>1.27 (1.28–3.69)</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>min PInteraction</td>
<td>0</td>
<td>0</td>
<td>1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc + smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/smoking (−)</td>
<td>320 (244)</td>
<td>348 (53.05)</td>
<td>320 (47.06)</td>
<td>1 (ref.)</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Anti-HBc(−)/smoking (+)</td>
<td>55 (46)</td>
<td>50 (7.62)</td>
<td>1.56 (0.98–2.49)</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/smoking (−)</td>
<td>269 (225)</td>
<td>215 (32.77)</td>
<td>1.41 (112–179)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>min PInteraction</td>
<td>0</td>
<td>0</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc + EBV antibodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc(−)/EBV antibodies (−)</td>
<td>694 (554)</td>
<td>680</td>
<td>0.92</td>
<td>0.87 (0.45–1.74)</td>
<td>0.69</td>
</tr>
<tr>
<td>Anti-HBc(−)/EBV antibodies (+)</td>
<td>14 (9)</td>
<td>313 (46.03)</td>
<td>1.44 (1.00–2.01)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>min PInteraction</td>
<td>0</td>
<td>0</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PInteraction</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AORs were adjusted for sex, age (as a continuous variable), anti-HBc, alcohol drinking, cigarette smoking, AST concentration, ALT concentration, and family history of cancer in all cases and benign tumor controls.

bAORs were adjusted for sex, age (as a continuous variable), anti-HBc, alcohol drinking, cigarette smoking, AST concentration, ALT concentration, family history of cancer, and EBV infection in the Cantonese cases and healthy population controls.

Department statistics by liver function, smoking, family history of cancer, and EBV infection between the association of anti-HBc and the risk of NPC with stratified analyses.

Discussion

On the basis of a large-scale case–control study, we are the first to report that HBV infection with anti-HBc(−) might be associated with an increased risk of NPC development in Southern China. The association was independent of a handful of potential confounders, including age, sex, family history of cancer, cigarette smoking, alcohol drinking, liver function, and EBV infection. Furthermore, we confirmed in the present study the previous findings that family history of cancer (35, 36) and EBV seropositivity (37) were both strong risk factors for NPC. Moreover, we for the first time reported that an abnormal ALT concentration might also be associated with a higher risk of NPC.

The potential mechanisms underlying the association between HBV infection and risk of NPC are unknown. To date, there is little evidence supporting the presence of HBV DNA and HBV antigen in the epithelial cells of the nasopharynx. Therefore, the potential role of HBV infection in the etiology of NPC might be indirect. For example, it is plausible that HBV interacts with EBV to induce and promote the pathogenesis of NPC.

Additionally, EBV is in latent status in B lymphocytes under the strict monitoring of the immune system (24). B lymphocytes can however be activated when infected with HBV, potentially activating or reactivating the latent EBV (22, 23). Specifically, the activated lymphocytes may increase virus virion shed from the lymphocytes to the nasopharynx. Furthermore, the proliferated B lymphocytes infected with EBV could also efficiently mediate the cell-to-cell contact mode for EBV infection in the epithelial cells (25). The efficiency of EBV infection in the epithelial cells through the cell-to-cell mode has been suggested to increase by 103 to 104 times, compared with the direct infection method using cell-free supernatants harvested from EBV-producing lymphoblast cells (38).
Subsequent systemic chronic inflammation and the altered cytokine network following chronic HBV infection might also be relevant in NPC development (39). Although it is yet to be shown to what extent chronic inflammation affects NPC development, histologically, NPC usually demonstrates as an inflammation-like lymphoepithelial carcinoma (26). Under a chronic inflammatory condition, the reactive oxygen and nitrogen species generated from inflammatory tissues may result in DNA damage and initiate gene mutation of nasopharyngeal epithelial cells (40). More evidence needs to be gathered in this regard, for example, through examining whether the risk of NPC is increased among patients of rhinosinusitis (41) and allergic rhinitis (42, 43).

Using never exposure to HBV as the reference group, our data showed that individuals with previous infection had similarly increased risk for NPC as those with chronic infection. The results largely persisted after further adjusting for EBV status, although the association for chronic infection became statistically nonsignificant potentially due to the relatively smaller sample size. The importance of previous and chronic HBV infection may highlight the potentially long induction period of HBV infection in promoting NPC development, whether or not in interaction with EBV. HBV infection in early life, typically during childhood, may additionally stand for exposure to other environmental risk factors for NPC, including salted fish, pickled food, and less access to fresh fruits. Childhood exposure to these risk factors may be more strongly related to NPC compared with adulthood exposure (44, 45).

The underlying reason for the lack of detectable HBsAg in patients with previous HBV infection is unclear, although host immune response, virus interference, epigenetic factors, etc., might all be important (46). After HBsAg disappearing, the systemic inflammation and HBV DNA may still persist in the body of the previous infection individuals and forced the body to react abnormally (47–50). As a result, anti-HBC may potentially be of more clinical importance, relative to other HBV biomarkers, concerning the need of understanding the link between HBV infection and future cancer development.

Serum ALT level is commonly used to measure the degree of HBV-related hepatocellular inflammation (28). ALT has been proven as an independent risk predictor for hepatocellular carcinoma (51) and may also be associated with pancreatic cancer (52) and non–Hodgkin lymphoma (53). In the present study, we showed that an abnormal ALT concentration might also be a risk factor for NPC, independent of HBV infection. The association may lend some support to the hypothesis regarding a role of chronic systematic inflammation in the development of NPC; however, the elevated ALT might also be a result of NPC, demonstrating the metabolic abnormalities or altered immunomodulation in a severe and chronic disease as NPC (54). Given the cross-sectional design of the present study, we were unable to disentangle whether abnormal ALT precedes or follows NPC. Further prospective studies should be encouraged to specifically examine the temporal relationship between liver function and NPC.

There was no statistically significant interaction of anti-HBC with ALT concentration, cigarette smoking, family history of cancer, or EBV serological antibodies, although the AORs were in general greater among individuals with anti-HBC(+) and also one of these risk factors. Given the relatively small numbers of individuals observed in each combination category of the present study, a real interaction between anti-HBC and these factors, especially EBV infection, could not be ruled out still.

We included a group of hospital controls (benign tumor controls) and a group of healthy population controls in the present study. Such a design with two control groups has the advantage of reducing potential selection bias when only "sick controls" or "healthy controls" are used in case–control studies (55, 56). Similar results obtained using these two types of controls further strengthened our conclusion. The prevalence of HBsAg positivity was 12.65% among the benign tumor controls and 14.26% among the healthy controls, similar to the general population of Southern China (32), indicating that both groups were representative of the source population for NPC cases in terms of HBV infection.

A few limitations of the present study should also be addressed. First, the interaction of HBV infection and EBV markers was only assessed in the analysis of NPC cases and healthy controls, as measurements for EBV markers were not available in benign tumor controls. Second, the association of cigarette smoking with NPC appeared to be inconsistent between using the benign tumor controls and healthy controls, indicating potential selection force by smoking in either group. Compared with the benign tumor controls, healthy controls were more likely to be smokers and had lower socioeconomic status (data not shown). It is known that the prevalence of smoking is higher in rural areas compared with urban areas in China (57). However, the largely similar results obtained when comparing NPC cases to either of the control groups indicated that the impact of this selection bias might be minimal. Third, we used different methods when collecting information on NPC risk factors, including smoking, drinking, and family history for cancer, namely, that information for cases and benign tumor controls was collected from medical records, whereas for healthy controls from in-person interview. In a post hoc validation study including 60 NPC cases diagnosed in the SYSUCC during 1 to 20, 2015, we found that the agreements for the variables of smoking, drinking, and family history of cancer between these two methods were high (all of proportions of agreement > 0.9). Finally, ALT and AST concentration in healthy population controls were measured retrospectively using long-term stored samples (collected in 2008 and assayed in 2014). We retested the ALT and AST concentration using 82 subjects to compare the difference between the values tested in 2008 and tested in 2014. After that, we adjusted the values of ALT and AST according the difference, respectively.

In conclusion, on the basis of a large case–control study of NPC in Southern China, we found that HBV infection was associated with a higher risk of NPC. Future prospective studies, including the analysis of HBV DNA load, are warranted to confirm these findings and to shed more light on the underlying mechanisms for these findings.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


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