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Evaluation of risk factors for extrahepatic cholangiocarcinoma: ABO blood group, hepatitis B virus and their synergism

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Little is known about the role of association between ABO blood group and development of extrahepatic cholangiocarcinoma (ECC) through effects on hepatitis B viral (HBV) infection. Our aim was to address this question using a matched case–control study in Southern China.We prospectively analyzed 239 ECC patients, and 478 age- and sex-matched controls in Sun Yat-sen Memorial Hospital of Sun Yat-sen University from 1999 to 2011. Information on ABO blood group, HBV infection and other clinicopathologic factors was collected. Adjusted odds ratios (AORs) and the corresponding 95% confidence intervals (CIs) were computed from unconditional logistic regression models, adjusted for major confounding factors. The estimated AORs were as follows: A blood group, 1.784; HBsAg+/HbcAb+, 1.848 and HBsAg−/HbcAb+, 1.501. The A blood type had a significant effect on modifying the risk of ECC among subjects with HBsAg+/HbcAb+ (AOR 3.795, 95% CI 1.427–10.090). ECC patients with A blood group were more common in younger subjects, and a lower proportion of serum CA-125 and CA19-9 elevation in patients with blood type A was found. Our study suggests an association between A blood type, HBV infection and ECC risk, and a synergism between A blood type and HBV infection in the development of ECC.

Key words: bile duct cancer, epidemiology, hepatitis, ABO blood group

Abbreviations: AOR: adjusted odds ratio; CC: cholangiocarcinoma; CI: confidence interval; ECC: extrahepatic cholangiocarcinoma; HBcAb: hepatitis B core antibody; HBeAb: hepatitis B e antibody; HBeAg: hepatitis B e antigen; HBsAb: hepatitis B surface antibody; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; ICC: intrahepatic cholangiocarcinoma; OR: odds ratio

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Cholangiocarcinoma (CC) is a malignant neoplasm of the biliary-duct system accounting for 10–25% of the primary hepatic malignancies worldwide.[1] Recent study showed that the incidence and mortality rates of CC have been increasing worldwide including China.[2–6] Although several risk factors for CC, including parasitic infection, primary sclerosing cholangitis, anatomical abnormalities and hepatolithiasis, have been established, some potential factors such as hepatitis virus infection and host genetic polymorphisms remain debating.[7] Meanwhile, data showed that potential risk factors might have different effect on CC, depending on the anatomical site.[8]

The role of HBV in ECC is not entirely clear, and the related studies have different conclusions.[7] The prevalence rate of infection with hepatitis B virus (HBV) varies widely among different parts of the world; it can range from near zero to more than 10%.[9] China is a typical highly endemic area of HBV infection. The effect of HBV infection on the incidence, age or sex distribution and clinicopathologic parameters of intrahepatic cholangiocarcinoma (ICC) has been documented by many studies, but the role of HBV in the development of extrahepatic cholangiocarcinoma (ECC) in viral hepatitis B endemic areas was less reported and remains to be addressed.

Furthermore, several genome-wide studies demonstrated that ABO blood group antigens may alter the systemic inflammatory response. And, blood antigens are also known

What's new?

Hepatitis B virus (HBV) infection is a suspected risk factor for extrahepatic cholangiocarcinoma (ECC), though how it gives rise to malignant disease of the bile ducts situated outside the liver is unclear. Here, a significant association was found for HBV infection, A blood type, and ECC risk in the Han Chinese ethnic group of Southern China. Compared with non-A blood group ECC patients, type A ECC patients tended to be younger and had lower CA-125 and CA19-9 levels. The study is the first to confirm an association between A blood group, HBV, and ECC risk.

to be important as receptors or ligands for microbes and immunologically important proteins.[10–12] The relationship between ABO blood group antigens and tumors, including stomach, breast, pancreas, ovary and skin, has been suggested by many investigations,[13–16] but the association of ABO blood group with the risk of ECC was not studied before. It was hypothesized that variations in immune response to infectious agents are often associated with several host factors. In fact, there has been a large amount of studies published on the chemistry of blood group antigens, tumor immunology and infectious disease.[17] Therefore, it is reasonable to make the hypothesis that variations in immune and inflammation response to HBV infection may be associated with ABO blood groups.

We performed a hospital based case–control study of ECC in the Han Chinese ethnic group of Southern China, in order to: (*i*) investigate the association of the two main controversial risk factors including HBV infection and ABO blood group with risk of ECC, (*ii*) evaluate the effect of HBV and ABO blood group on modifying clinicopathological characteristics of ECC and (*iii*) explore the synergism between ABO blood type and HBV infection in the development of ECC.

Methods

Study population

We performed a manual retrospective review of the patients' medical records. Medical records were identified for CC patients with pathological diagnosis referred to the Sun Yat-sen Memorial Hospital of Sun Yat-sen University from January 1, 1999, through December 31, 2011. The anatomic location of the tumor was confirmed again by review of radiology results (ultrasound, computerized tomography, magnetic resonance imaging or endoscopic retrograde cholangiopancreatography) and/or surgical operation records. CC was classified as either "intrahepatic" or "extrahepatic" depending on those results. ECC includes perihilar CCs (also called Klastskin tumors), which occur at or near the junction of the right and left hepatic ducts and the common bile duct tumors arising in the extrahepatic bile ducts above the superior border of the pancreas. The vater ampulla carcinoma was excluded in this analysis.

Using the Statistical Package for Social Science Program (SPSS, version 16, Chicago, IL), we randomly selected hospital-based control groups for ECC cases at a ratio of 2:1 from the hospital electronic medical record system. This system includes all the medical records of inpatients since 1991. Eligible controls should meet the following criteria: (*i*) patients without digestive tract disease; (*ii*) those were admitted for acute non-neoplastic conditions with histological confirmation; (*iii*) those also received medical checkup (including fecal occult blood tests, serum tumor marker test, abdominal ultrasound, abdominal computerized tomography and/or magnetic resonance imaging and chest radiograph) for excluding other potential malignant diseases and (*iv*) without known history of malignant disease. From subjects who meet the criteria, we randomly selected 478 age- and sex-matched controls. The main diagnoses of those controls who were included in this analysis were diseases of the nervous system (19.5%), musculoskeletal system and connective tissue (19.5%), injuries and other external causes (18.8%), genitourinary system (9.8%), respiratory system (5.0%) and other conditions (27.4%). The same information and risk factor data as the study cases from medical records were collected.

Laboratory test for HBV infection and ABO blood group

HBV serologic test is a conventional event for tumor patients at their initial visit to the two hospitals. An enzyme-linked immunosorbent assay (ELISA) was used to test for the presence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HbsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb) and hepatitis B core antibody (HBcAb). The ABO blood groups (A, B, O and AB) were examined using mouse-derived monoclonal antibodies or ELISA. All the tests were performed in the clinical laboratories of each hospital. The definition of various HBV test results is as follows: briefly, all five negative results were defined as never exposed to HBV; patients with only HBsAb positive were defined as those who acquired artificial immunization or previously exposed to HBV with immunity; patients with HBsAg positive were considered as chronic carriers, and patients with HBcAb positive but HBsAg negative were defined as asymptomatic carrier.

Definition of liver cirrhosis

The diagnostic criteria for cirrhosis were as follows: clinical manifestations or radiologic evidence of portal hypertension (*e.g.*, collateral varices, varices, thrombocytopenia or splenomegaly) and/or impaired hepatic function, radiologic evidence of a nodular liver and caudate lobe hypertrophy. For patients with surgical treatment, pathology results that reported liver character were also criteria.

Table 1. Baseline characteristics of cases with extrahepatic cholangiocarcinoma and controls without cancer

Variable	Cases, $N = 239$	Controls, $N = 478$	p-Value
Age, mean ± SD[1]	61.6 ± 9.8	60.9 ± 10.0	0.493
Gender[2]			
Male	156	312	1.000
Female	83	166	
HBV serologic results, n (%)[2]	$N = 222$	$N = 478$	
HBsAg−/HBcAb−	132 (59.5)	330 (69.0)	
HBsAg+	27 (12.2)	45 (9.4)	0.123
HBsAb+	122 (55.0)	261 (54.6)	0.300
HbeAg+	2 (0.9)	4 (0.8)	0.932
HbeAb+	45 (20.3)	101 (21.1)	0.833
HBcAb+	88 (39.6)	139 (29.1)	0.007
HBsAg+/HBcAb+	25 (11.3)	36 (7.5)	0.047
HBsAg−/HBcAb+	63 (24.8)	103 (21.5)	0.025

[1]Independent sample t-test.
[2]Pearson's χ^2 test.
Abbreviations: HBsAg: hepatitis B surface antigen; HBsAb: hepatitis B surface antibody; HBeAg: hepatitis B e antigen; HBeAb: hepatitis B e antibody; HBcAb: hepatitis B core antibody; "+": positive results; "−": negative results.

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Science Program (SPSS). The p value <0.05 (two-tailed) was considered to be statistically significant. The Pearson's χ^2 test was used to compare categorical variables, and quantitative variables were analyzed by independent two-sample t-test. Potential risk factors of individuals in the study group and the control group were compared by using univariate logistic regression analysis. The significant variables in univariate logistic regression were further adjusted for the matching factors (age and sex) to be tested in unconditional multiple logistic regression. We estimated odds ratio (OR), adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for each factor by the maximum likelihood method.

Results

Patient characteristics

Two hundred and thirty-nine ECC cases were included in this analysis, and 478 controls were selected. One hundred and thirty-six patients in the ECC group (56.9%) had Klatskin tumors. The ECC cases involved 156 males and 83 females, ranging from 22 to 83 years of age (mean ± standard deviation, 61.6 ± 9.8). The mean age of controls was 60.9 ± 10.0 years. Because cases and control subjects were matched for sex and age, there were no differences in the distributions of these two baseline features between cases and controls. In addition, there were no significant differences in blood group or HBV serological markers among two groups (Table 1).

Univariate analysis of the risk factors for ECC

As shown in Table 2, univariate analysis showed that HBV infection was significantly associated with an increased risk of ECC (HbcAb+: OR, 1.583; 95% CI, 1.133–2.212; $p = 0.007$; HBsAg+/HBcAb+: OR, 1.736; 95% CI, 1.003–3.005; $p = 0.049$ and HBsAg−/HBcAb+: OR, 1.529; 95% CI, 1.053–2.220; $p = 0.026$). It also showed that percentage of A blood group was significantly higher in ECC patients than controls (34.3 *vs.* 24.9%; OR, 1.646; 95% CI, 1.127–2.403; $p = 0.010$).

Besides, ECC patients had a higher prevalence of liver cirrhosis (9.2 *vs.* 2.1%, $p < 0.001$), cholecystolithiasis (13.4 *vs.* 4.0%, $p < 0.001$), choledocholithiasis (14.2 *vs.* 1.3%, $p < 0.001$), hepatolithiasis (7.9 *vs.* 1.7%, $p < 0.001$), history of cholecystectomy (6.3 *vs.* 1.7%, $p = 0.016$), diabetes mellitus (12.1 *vs.* 6.3%, $p = 0.008$) and family history of other cancer (8.8 *vs.* 3.1%, $p = 0.002$).

Multivariate analysis

Table 2 shows the multivariate-adjusted risk factors discovered in the univariate analysis. HBV infection (HBsAg+/HBcAb+: AOR, 1.848; 95% CI, 1.039–3.289; $p = 0.037$ and HBsAg−/HBcAb+: AOR, 1.501; 95% CI, 1.004–2.245; $p = 0.048$), A blood group (AOR, 1.784; 95% CI, 1.172–2.714; $p = 0.007$), liver cirrhosis (AOR, 3.301; 95% CI, 1.418–7.684; $p = 0.006$), cholecystolithiasis (AOR, 3.889; 95% CI, 2.076–7.286; $p < 0.001$), choledocholithiasis (AOR, 9.849; 95% CI, 3.093–24.854; $p \leq 0.001$), hepatolithiasis (AOR, 3.091; 95% CI, 1.193–8.004; $p = 0.020$), history of cholecystectomy (AOR, 4.040; 95% CI, 1.583–10.311; $p = 0.003$), diabetes mellitus (AOR, 1.960; 95% CI, 1.074–3.578; $p = 0.028$) and family history of other cancer (AOR, 3.155; 95% CI, 1.552–6.411, $p = 0.001$).

The association between blood group and ECC risk was further evaluated among the patients with HBV test results, after additional adjusting for HBV infection (HBsAg+/HBcAb+). The analysis also showed a statistically significant increased risk of ECC among persons with A blood type (AOR, 1.801; 95% CI, 1.181–2.743; $p = 0.006$).

Sex and age distributions of ECC patients depending on HBV or ABO blood group

As shown in Table 3, ECC showed a statistically significant younger onset age in patients with HBV infection (58.8 ± 9.2 *vs.* 62.8 ± 10.0, $p = 0.026$). In addition, HBV-related ECC patients were more common in male and subjects (male/female, 33/7; $p = 0.037$).

The younger onset age of ICC patients with HBV infection compared to ICC patients without HBV infection has been documented, but the definition of HBV infection in these studies was different, and it was little studied in ECC. Hence, to further research the details about onset age, we

Table 2. Univariate (crude) and multivariate (adjusted) analysis for risk factors for extrahepatic cholangiocellular carcinoma

	Cases (%)		Controls (%)		Univariate			Multivariate		
Variable	No.	%	No.	%	OR	95% CI	*p*-Value	OR[1]	95% CI	*p*-Value
ABO blood type	239		478							
O	85	35.6	203	42.5		1 (reference)			1 (reference)	
A	82	34.3	119	24.9	1.646	1.127–2.403	0.010	1.784, 1.801[2]	1.172–2.714, 1.182–2.743[2]	0.007, 0.006[2]
B	63	26.4	115	24.1	1.308	0.879–1.948	0.186	1.270	0.815–1.981	0.291
AB	9	3.8	41	8.6	0.524	0.244–1.126	0.098	0.445	0.177–1.119	0.085
A + AB	91	38.1	160	33.5	1.358	0.947–1.949	0.096			
B + AB	72	30.1	156	32.6	1.102	0.756–1.607	0.613			
A + B + AB	154	64.4	275	57.5	1.337	0.970–1.844	0.076			
HBV	222[3]		478							
HBsAg−/HBcAb−	132	59.5	330	69.0		1 (reference)			1 (reference)	
HbsAg+	27	12.2	45	9.4	1.500	0.894–2.518	0.125			
HbsAb+	122	55.0	261	54.6	1.169	0.870–1.569	0.300			
HbeAg+	2	0.9	4	0.8	1.250	0.226–6.907	0.798			
HbeAb+	45	20.3	101	21.1	1.114	0.743–1.670	0.602			
HbcAb+	88	39.6	139	29.1	1.583	1.133–2.212	0.007			
HBsAg+/HBcAb+	25	11.3	36	7.5	1.736	1.003–3.005	0.049	1.848	1.039–3.289	0.037
HBsAg−/HBcAb+	63	28.4	103	21.5	1.529	1.053–2.220	0.026	1.501	1.004–2.245	0.048
Liver cirrhosis	239		478							
No	217	90.8	468	97.9		1 (reference)			1 (reference)	
Yes	22	9.2	10	2.1	4.745	2.209–10.193	<0.001	3.301	1.418–7.684	0.006
HBV-related cirrhosis	12	5.0	6	1.3	4.313	1.598–11.644	0.004	3.425	1.193–9.834	0.022
Other cirrhosis	10	4.2	4	0.8	5.392	1.672–17.383	0.005	6.168	1.846–20.612	0.003
Cholecystolithiasis	239		478							
No	207	86.6	459	96.0		1 (reference)			1 (reference)	
Yes	32	13.4	19	4.0	3.735	2.068–6.743	<0.001	3.889	2.076–7.286	<0.001
Choledocholithiasis	239		478							
No	205	85.8	472	98.7		1 (reference)			1 (reference)	
Yes	34	14.2	6	1.3	13.047	5.394–31.557	<0.001	9.849	3.093–24.854	<0.001
Hepatolithiasis	239		478							
No	220	92.1	470	98.3		1 (reference)			1 (reference)	
Yes	19	7.9	8	1.7	5.074	2.187–11.770	<0.001	3.091	1.193–8.004	0.020
Diabetes mellitus	239		478							
No	210	87.9	448	93.7		1 (reference)			1 (reference)	
Yes	29	12.1	30	6.3	2.062	1.206–3.525	0.008	1.960	1.074–3.578	0.028
Cigarette smoking	239		478							
No	179	74.9	383	80.1		1 (reference)			1 (reference)	
Past + current	60	25.1	95	19.9	1.351	0.935–1.954	0.109	1.301	0.863–1.962	0.209
Alcohol drinking	239		478							
No	192	80.3	397	83.1		1 (reference)			1 (reference)	
Yes	47	19.7	81	16.9	1.200	0.805–1.787	0.370	1.053	0.670–1.655	0.822
History of cholecystectomy	239		478							
No	224	93.7	470	98.3		1 (reference)			1 (reference)	
Yes	15	6.3	8	1.7	3.934	1.644–9.416	0.002	4.040	1.583–10.311	0.003

Table 2. Univariate (crude) and multivariate (adjusted) analysis for risk factors for extrahepatic cholangiocellular carcinoma (Continued)

	Cases (%)		Controls (%)		Univariate			Multivariate		
Variable	No.	%	No.	%	OR	95% CI	*p*-Value	OR[1]	95% CI	*p*-Value
First-degree relatives of cholangiocarcinoma	239		478							
No	238	99.6	478	100.0		1 (reference)			1 (reference)	
Yes	1	0.4	0	0.0	—	—	1.000	—	—	1.000
Family history of other cancer	239	100.0	478							
No	218	91.2	463	96.9		1 (reference)			1 (reference)	
Yes	21	8.8	15	3.1	2.973	1.504–5.880	0.002	3.155	1.552–6.411	0.001

[1]The OR was adjusted by sex, age (as continuous variable), liver cirrhosis, cholecystolithiasis, choledocholithiasis, hepatolithiasis, history of cholecystectomy, diabetes mellitus and family history of other cancer.
[2]The OR was adjusted by HBV infection, sex, age (as continuous variable), liver cirrhosis, cholecystolithiasis, choledocholithiasis, hepatolithiasis, history of cholecystectomy, diabetes mellitus and family history of other cancer.
[3]Seventeen ECC cases lacked HBV serologic test results.
Abbreviations: OR: odds ratio; 95% CI: 95% confidence interval; HBsAg: hepatitis B surface antigen; HBsAb: hepatitis B surface antibody; HBeAg: hepatitis B e antigen; HBeAb: hepatitis B e antibody; HBcAb: hepatitis B core antibody; "+": positive results; "−": negative results.

Table 3. Subgroup and among-group comparisons of age and sex distributions depending on HBV infection

	Infection state		
Events	HBV infection[1]	Noninfection[2]	*p*-Value
Gender			
Male (*n*)	33	86	0.037[3]
Female (*n*)	7	46	
Total (*n*)	40	132	
Age			
Median (range)	57.5 (40–81)	63 (39–80)	0.026[4]
Mean ± SD	58.8 ± 9.2	62.8 ± 10.0	

[1]HBV infection patients = HBsAg+/HBcAb+ or HBsAb−/HBcAb+ patients, representing patients with previous exposure to HBV but without immunity.
[2]Non-HBV infection patients = HBsAg−/HBcAb− patients, representing patients without exposure to HBV or with immunity.
[3]Pearson's χ^2 test.
[4]Student's *t*-test.

directly evaluated age distribution depending on different hepatitis B serologic results. Two serologic results, HBsAg+ ($p = 0.026$; Kruskal–Wallis test) and HBsAg+/HBcAb+ ($p = 0.033$; Kruskal–Wallis test), were both identified to be associated with the young onset age of ECC.

As A blood group was found to be significantly associated with an increased risk of ECC in the multivariate analysis, to further clarify the characteristics of ECC with A blood group, the age and sex distributions were compared between ECC patients with blood type A and those with other blood types (Table 4). The mean age of ECC patients with A blood group was 2.7 younger than that of non-A blood type ECC patients, and the difference was statistically significant (59.9 ± 10.6 *vs.* 62.6 ± 10.0, $p = 0.050$). The differences of sex composition were not significant between A blood group patients and others.

Clinicopathological characteristics of ECC patients with HBV infection or A blood group

Tumor marker tests were performed preoperatively as routine for malignance patients in our hospital. As shown in Table 5, compared to ECC patients without HBV infection, patients with HBV infection had a higher prevalence of abnormal CA-125 level (60 *vs.* 31.8%, $p = 0.007$) and lower abnormal CA-199 value (60 *vs.* 82.6%, $p = 0.011$). In addition, a lower proportion of abnormal CA-125 (25.6 *vs.* 38.9%, $p = 0.041$) or CA19-9 (67.1 *vs.* 84.7%, $p = 0.002$) value was found in A blood group patients (67.1 *vs.* 84.7%, $p = 0.002$). The incidences of other events, including advanced T stage, poor histological grade, lymph node involvement, neural invasion and vascular infiltration, were not correlated with HBV infection or ABO blood group.

Synergistic effect between HBV and ABO blood group

Table 6 shows the association between A blood group, HBV infection and ECC risk. The multivariate analysis, after adjusting for liver cirrhosis, cholecystolithiasis, choledocholithiasis, hepatolithiasis, history of cholecystectomy and family history of other cancer, revealed a greatest ECC risk for blood type A patients with both HBsAg positive and HBcAb positive (AOR, 3.795; 95% CI, 1.427–10.090; $p = 0.008$). Among the non-A blood group patients, compared to those with both HBsAg negative and HBcAb negative, patients with seropositive results for HBsAg and HBcAb did not show a significant increased ECC risk (AOR, 1.594; 95% CI, 0.768–3.308; $p = 0.211$).

In addition, among patients with HBsAg positive, multivariate analysis showed that blood type A patients had a greater ECC risk than non-A blood group cases (2.701 *vs.* 1.079). However, in silent HBV carriers (HBsAg−/HBcAb+), those with A blood group did not have a higher ECC risk than non-A blood group ones (1.495 *vs.* 1.958). These

Table 4. Comparison of age between A blood group patients *vs.* non-A blood group or O blood group

Events	A blood group	Non-A blood groups			
Age	A	O	B + O	AB + O	B + AB + O
Cases (*n*)	82	85	148	94	157
Median	59.5 (22–80)	65.5 (39–81)	63 (39–82)	64.5 (39–81)	63 (35–82)
Mean ± SD	59.9 ± 10.6	63.2 ± 9.8	62.6 ± 10.1	63.1 ± 9.5	62.6 ± 10.0
p-Value[1]	Reference	0.033	0.049	0.035	0.050
Gender	A	O	B + O	AB + O	B + AB + O
Cases (*n*)	82	85	148	94	157
Male (*n*)	58	53	91	60	98
Female (*n*)	24	32	57	34	59
p-Value[2]	Reference	0.252	0.160	0.331	0.200

[1]Student's *t*-test.
[2]Pearson's χ^2 test.

findings indicate that there may be a synergism between A blood type and HBV infection in the development of ECC among HBsAg-positive patients, especially those who have both serologic HBsAg-positive and HBcAb-positive test results. Furthermore, well-designed and larger sample size studies or meta-analysis are needed to further clarify whether this synergism effect exists in silent HBV carriers.

Discussion

To our knowledge, our study is the first analysis studying the association between ABO blood group and HBV infection and ECC risk. Overall, our study provides evidence in support of the association of ECC with HBV infection or A blood group and suggests a synergism between A blood type and HBV infection in the development of ECC. In addition, we also evaluated the effect of these two risk factors on the distribution of age, sex and other clinicopathologic factors. The analysis confirmed that both HBV infection and A blood group had a modified effect on early onset age and male susceptibility. Besides, we also found a lower proportion of serum CA19-9 and CA-125 elevation in A blood group patients; therefore, caution is suggested when interpreting the serum CA-125 or CA 19-9 levels in A blood group patients who are suspected to suffer ECC.

It is known that ABO and Lewis blood group antigens belong to cell surface carbohydrate structures, and the relationship between cell surface carbohydrate structures and the biological behavior of tumors has been described previously.[18,19] Structural changes in ABO antigens during tumorigenesis have been demonstrated; the ABO antigens on cell surfaces act as important mediators of intercellular adhesion and membrane signaling, which are integral to malignant progression and spread,[20] but the exact mechanisms by which the expressions of certain carbohydrate structures influence tumor progression remain incomplete. In the non-O blood group, deletion of A or B antigens induces upregulation of precursor H and Lewis^y expression, and both precursors exert angiogenic effects.[21] Individuals with non-O blood type also have a markedly increased risk of thrombotic and hemorrhagic events.[22] Both effects may facilitate hematogenous metastasis.

Although clinicopathologic characteristic of ABO blood types was investigated for patients with kinds of cancer,[23–25] there is little information available regarding the relationship between ABO blood type and oncological characteristic of CC. In this regard, the prominent findings of our study were lower proportion of serum CA19-9 and CA-125 elevation in patients with blood type A. Because our analysis also revealed that blood type A was associated with a significant increased risk for ECC, caution is suggested when interpreting the serum CA-125 or CA 19-9 levels in patients with A blood group.

The mechanism by which HBV may induce ECC has not yet been elucidated in detail. However, the findings that HBV DNA, proteins and hepatitis C virus RNA were detected in tissue specimens from both intrahepatic and extrahepatic bile duct cancers indicate that HBV may play a role in tumorigenesis.[26–28] Recently, several studies suggested that both HBV-related ICC and hepatocellular carcinoma (HCC) held common disease processes for carcinogenesis, and may originate from hepatic progenitor cells.[29–32] As evidence, proportion of AFP elevation was far higher in HBV-related ICC when compared to ICC with HBsAg negative.[32,33] However, our study showed that AFP was in the normal level for most ECC cases, regardless of the HBV infection state. This finding was quite different from that in ICC patients, which suggested a different role of HBV in the development of ECC. More recently, two studies identified that biliary tree stem/progenitors cells, present in peribiliary glands of extrahepatic biliary trees including pancreas, are likely to be central to normal tissue turnover and injury repair and to be key elements in the pathophysiology of liver, pancreas and biliary tree diseases, including oncogenesis.[34,35] These studies indicated a new carcinogenic target of HBV infection. Further

Table 5. Clinicopathologic characteristic of patients with HBV infection or A blood group, compared to patients without HBV infection or non-A blood group

	HBsAg+/ HBcAb+		HBsAg−/ HBcAb−			A		B/AB/O		
Characteristic	*n*	%	*n*	%	p^1	*n*	%	*n*	%	p^1
AFP[2]					0.405					0.692
≤25	24	96.0	130	98.5		81	98.8	154	98.1	
>25	1	4.0	2	1.5		1	1.2	3	1.9	
CEA[2]					0.291					0.540
≤5	19	76.0	86	65.2		56	68.3	101	64.3	
>5	6	24.0	46	34.8		26	31.7	56	35.7	
CA-125[2]					0.007					0.274
≤35	10	40.0	90	68.2		61	74.4	96	61.1	0.041
>35	15	60.0	42	31.8		21	25.6	61	38.9	
CA19-9[2]					0.011					0.002
≤35	10	40.0	23	17.4		27	32.9	24	15.3	
>35	15	60.0	109	82.6		55	67.1	133	84.7	
Primary tumor					0.295					0.580
T1 or T2	4	16.0	17	12.9		12	14.6	19	12.1	
T3 or T4	21	84.0	115	87.1		70	85.4	138	87.9	
Histological grade					0.627					0.583
Well or moderate	16	64.0	91	68.9		53	64.6	107	68.2	
Poor	9	36.0	41	31.1		29	35.4	50	31.8	
Lymph node involvement					0.862					0.849
Yes	7	35.0	40	37.0		26	37.7	50	39.1	
No	13	65.0	68	63.0		43	62.3	78	60.9	
Neural invasion					0.707					0.309
Yes	3	15.0	20	18.5		16	23.2	22	17.2	
No	17	85.0	88	81.5		53	76.8	106	82.8	
Vascular infiltration					0.526					0.719
Yes	3	15.0	11	10.2		7	10.1	11	8.6	
No	17	85.0	97	89.8		62	89.9	117	91.4	

[1]Pearson's χ^2 test.
[2]The cutoff values were based on the normal reference range in our laboratory.

studies are needed to explore the role of stem cells and its interaction with HBV in the development of ECC.

It has been well documented that men are more likely to develop HCC compared to women.[36] Both androgen and estrogen have been reported to be associated with development of HCC.[37–39] Our analyses revealed that the risk of HBV-associated ECC was also higher in men than in women. However, it remains unknown what role sex hormones play in the development of ECC, and what interactive mechanism between hormones and HBV exists. Besides modified effect on sex distribution, our study also confirmed a statically significant advanced onset age for HBV-related ECC patients. This is the first study evaluating the modified effect of HBV on onset age in ECC.

The synergism between ABO blood group and HBV infection revealed by our analysis may be explained by several biological effects of blood group. First, several genome-wide studies have demonstrated that genetic variation in the first intron of ABO genes may alter the systemic inflammatory response, by regulating circulation levels of serum tumor necrosis factor alpha, soluble intracellular adhesion molecule and plasma levels of alkaline phosphatase.[10–12] For example, levels of soluble form of intercellular adhesion molecule-1 (sICAM-1) are significantly decreased in patients with blood groups A and B (in particular blood group A), and decreased serum concentrations of sICAM-1 have been implicated with increased inflammatory conditions.[40] Second, blood antigens are now known to be important as receptors or ligands for microbes

Table 6. Association between hepatitis B virus infection and A blood group with risk of ECC, including 222 patients with HBV serologic results

	Cases (%)		Controls (%)		Univariable			Multivariable		
Risk factors	No.	%	No.	%	OR	95% CI	*p*-Value	OR[1]	95% CI	*p*-Value
A blood type and HBsAg	222		478							
Non-A blood type/HBsAg−	131	59.0	325	66.7		1 (reference)				
A blood type/HBsAg−	64	28.8	108	23.0	1.470	1.016–2.128	0.041	1.557	1.043–2.323	0.030
Non-A blood type/HBsAg+	15	6.8	35	7.3	1.063	0.562–2.012	0.851	1.189	0.605–2.337	0.615
A blood type/HBsAg+	12	5.4	10	2.7	2.977	1.256–7.095	0.013	3.044	1.242–7.458	0.015
A blood type and HBsAb	222		478							
Non-A blood type/HBsAb−	59	26.6	165	36.6		1 (reference)				
A blood type/HBsAb−	41	18.5	52	10.9	2.205	1.330–3.657	0.002	2.882	1.651–5.034	0.001
Non-A blood type/HBsAb+	87	39.2	190	37.7	1.281	0.866–1.893	0.215	1.425	0.934–2.176	0.101
A blood type/HBsAb+	35	15.8	71	14.9	1.379	0.843–2.278	0.210	1.494	0.871–2.562	0.145
A blood type and HBcAb	222		478							
Non-A blood type/HBcAb−	83	37.4	256	51.9		1 (reference)				
A blood type/HBcAb−	51	23.0	83	17.6	1.895	1.236–2.907	0.003	2.064	1.300–3.276	0.002
Non-A blood type/HBcAb+	63	28.4	103	22.4	1.887	1.265–2.813	0.002	1.851	1.201–2.855	0.005
A blood type/HBcAb+	25	11.3	36	8.2	2.142	1.215–3.777	0.008	2.076	1.125–3.829	0.019
A blood type and HBsAg/HBcAb	222		478							
Non-A blood type/ HBsAg−/HBcAb−	82	36.9	249	50.8		1 (reference)				
A blood type/HBsAg−/ HBcAb−	50	22.5	81	17.2	1.874	1.217–2.887	0.004	2.017	1.267–3.214	0.003
Non-A blood type/HBsAg+/ HBcAb+	14	6.3	27	6.3	1.575	0.788–3.146	0.199	1.594	0.768–3.308	0.211
A blood type/HBsAg+/ HBcAb+	11	5.0	9	2.3	3.711	1.486–9.272	0.005	3.795	1.427–10.090	0.008
Non-A blood type/HBsAg−/ HBcAb+	49	6.3	76	6.3	1.958	1.264–3.032	0.003	1.958	1.207–3.176	0.007
A blood type/HBsAg−/ HBcAb+	14	5.0	27	2.3	1.575	0.788–3.146	0.199	1.495	0.703–3.178	0.296

[1]The OR was adjusted by liver cirrhosis, cholecystolithiasis, choledocholithiasis, hepatolithiasis, history of cholecystectomy, diabetes mellitus and family history of other cancer.
Abbreviations: OR: odds ratio; 95% CI: 95% confidence interval; HBsAg: hepatitis B surface antigen; HBsAb: hepatitis B surface antibody; HBeAg: hepatitis B e antigen; HBeAb: hepatitis B e antibody; HBcAb: hepatitis B core antibody; "+": positive results; "−": negative results.

and immunologically important proteins.[41] It is a plausible hypothesis that ABO blood groups may influence HBV-related immune response *via* their receptor-mediated affinity binding.

Conclusion

In our study, to our knowledge, we revealed that A blood group was associated with an increased risk of ECC for the first time. We also confirmed the significant association between HBV infection and ECC in viral hepatitis B highly endemic areas. In addition, compared to non-A blood group ECC patients, those with A blood group showed a younger onset age and lower CA-125 and CA19-9 level. Most important, we observed a novel synergetic mechanism between blood types and HBV infection, and found that A blood type and seropositivity for HBsAg, especially for both HBsAg and HBcAb, but not only HBcAb, may increase the risk of developing ECC.

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