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ORIGINAL ARTICLE

Case Control Study

Hepatitis B virus infection, diabetes mellitus, and their synergism for cholangiocarcinoma development: A case-control study in Korea

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Abstract

AIM: To identify possible risk factors and their synergism for cholangiocarcinoma development.

METHODS: A hospital-based, case-control study in which we included 276 cholangiocarcinoma patients [193 extrahepatic cholangiocarcinoma (ECC) and 83 intrahepatic cholangiocarcinoma (ICC)], diagnosed at a training hospital in Korea between 2007 and 2013, and 552 healthy controls matched 2:1 for age, sex, and date of diagnosis. Risk factors for cholangiocarcinoma and possible synergism between those factors were evaluated using conditional logistic regression and synergism index, respectively.

RESULTS: There was an association between cholangiocarcinoma and hepatitis B virus (HBV) infection, diabetes mellitus (DM), cholecystolithiasis, choledocholithiasis, and hepatolithiasis, with the adjusted odds ratios (AORs) of 4.1, 2.6, 1.7, 12.4, and 39.9, respectively. Synergistic interaction on the additive model was investigated between HBV infection and DM (AOR = 12.2; 95%CI: 1.9-80.1). In the subgroup analyses, cholecystolithiasis, choledocholithiasis, hepatolithiasis, and DM were significant risk factors for ECC (AOR = 2.0, 18.1, 14.9, and 2.0, respectively), whereas choledocholithiasis, hepatolithiasis, HBV infection, and DM were risk factors for ICC (AOR = 8.6, 157.4, 5.3 and 4.9, respectively). Synergistic interaction was also observed between HBV infection and DM (OR = 22.7; 95%CI: 2.4-214.1). However, there was no synergistic interaction between other significant risk factors for cholangiocarcinoma.

CONCLUSION: In this Korean study, HBV infection and DM were found to exert independent and synergistic effects on the risk for cholangiocarcinoma, including ICC. Exploring the underlying mechanisms

for such synergy may lead to the development of cholangiocarcinoma prevention strategies in high-risk individuals.

Core tip: Although several risk factors for cholangiocarcinoma were identified in previous studies, details on their interactions or the influence of disease duration on the risk of cholangiocarcinoma are still unclear. Moreover, epidemiologic studies about cholangiocarcinoma in Korea are scarce. The present study in a Korean population showed that the impact of diabetes mellitus on the risk of cholangiocarcinoma was greater when diabetic complications were present. Further, it indicated that there was a synergistic effect between Hepatitis B virus infection and diabetes mellitus on the risk of cholangiocarcinoma, and that the synergistic effect was enhanced in cases of complicated diabetes.

INTRODUCTION

Cholangiocarcinomas (CCAs) are highly fatal cancers of the biliary tract epithelium, which arise from intrahepatic [intrahepatic cholangiocarcinoma (ICC)] or extrahepatic bile ducts [extrahepatic cholangiocarcinoma (ECC)]. Although a rare malignancy, CCA is the second most common cancer of the liver[1]. Furthermore, the incidence of CCA has reportedly been increasing in several areas worldwide, especially the incidence of intrahepatic CCA[2-4]. Most CCAs are unresectable at presentation. Even after curative resection, 5-year survival rates of only 11%-44% have been reported[1]. Considering the poor prognosis and increasing incidence, it is crucial to recognize risk factors for CCA in order to decrease its incidence.

Several risk factors, including liver fluke infestation[5] and hepatolithiasis[6,7], were identified in East Asia including Korea, where CCA is more prevalent than in Western countries[8]. However, those account for < 30% of all CCA cases[1]. Recently, hepatitis B virus (HBV) infection[9] and diabetes mellitus (DM)[10] have been reported to be additional possible risk factors, but it has been estimated that only < 25% of CCA cases are related to these factors[7,11].

For the other primary liver cancer such as hepatocellular carcinoma, several synergistic effects between risk factors have been identified[12,13]. However, there have been little studies to focus on analyzing interactions between risk factors for CCA. Because of the multifactorial nature of biliary tract carcinogenesis, possible interactions between risk factors may exist. Therefore, we conducted a hospital-based case-control study to assess potential risk factors for CCA in Korea, and further evaluate possible synergisms between the risk factors identified.

MATERIALS AND METHODS

Study population

All patients diagnosed with CCA through pathological findings at the Cheju Halla General Hospital between January 2007 and April 2013 were reviewed for study enrollment. Pathological confirmation was based on definite cytology, small biopsy, or surgical pathology. Individuals diagnosed with other cancers before the date of CCA diagnosis were excluded from enrollment.

Control subjects, matched 2:1 with cases for age (± 3 years), sex, and date of diagnosis (± 3 mo), were randomly chosen among individuals who had visited the health screening center of the Cheju Halla General Hospital for a routine checkup during the same period as the CCA cases. We excluded subjects with diagnoses of cancers or who were missing any data regarding risk factors and cancers. Subjects without radiologic informations were also excluded. Finally, 276 cases and 552 controls were included for the analysis. The study protocol was approved by the Institutional Review Board of Cheju Halla General Hospital.

Data collection

Cases and controls were interviewed at the initial visit on their medical history, smoking, and alcohol use. Structured data collection sheets were routinely used in health screening center to obtain data on demographic and clinical characteristics. All eligible participants underwent radiological evaluations (abdominal ultrasound, computed tomography, and/or magnetic resonance cholangiopancreatography). Blood samples were also collected from all subjects at the time of initial examination.

All variables investigated for CCA risk evaluation were divided into 4 broad categories: biliary tract conditions, infectious etiologies, non-infectious liver diseases, and miscellaneous potential risk factors. Biliary tract conditions included cholecystolithiasis, choledocholithiasis, hepatolithiasis, cholecystectomy, primary sclerosing cholangitis, choledochal cyst, and liver fluke infestation. Non-infectious liver diseases included non-specific liver cirrhosis and alcoholic hepatitis. The infectious diseases group included HBV infection and hepatitis C virus (HCV) infection. The miscellaneous potential risk factors included smoking, alcohol, obesity,

DM, thyroid disease, chronic pancreatitis, hypertension, and ulcerative colitis.

All data were obtained retrospectively from patient records. We only included information up to 1 year before the diagnosis of CCA for cases and 1 year before the cancer diagnosis of the index case for the matched controls.

Definitions of events
CCA was classified as either intrahepatic or extrahepatic CCA. Hilar CCA was included in ECC, and ampulla of Vater cancer was excluded in this analysis. A heavy drinker was defined as an individual currently drinking alcoholic beverages in a daily amount of $\geq$ 80 g (male) or $\geq$ 40 g (female)[14]. Obesity was defined as a body mass index of 25.0 kg/m^2 or greater, according to the Asian-Pacific criteria for obesity[15].

Blood samples were collected from cases and controls at the time of initial examination. Serum HBV surface antigen (HBsAg) and HCV antibody (anti-HCV) were assessed by using enzyme immunoassay (Abbott Laboratories, North Chicago, IL, United States), and anti-HCV-positive participants were tested for HCV RNA by using COBAS® Ampliprep (Roche Molecular Systems, Inc., CA, United States). HBV infection was defined as a positive hepatitis B surface antigen, and HCV infection was defined as a positive HCV RNA. The diagnostic criteria for cirrhosis were as follows: clinical manifestations of chronic hepatitis with portal hypertension (*e.g.*, collateral varices, varices, thrombocytopenia, or splenomegaly) and/or hepatic decompensation (*e.g.*, jaundice, prolonged prothrombin time, and ascites), laboratory tests, and radiologic studies. In patients undergoing surgical treatment, cirrhosis was also confirmed pathologically. Nonspecific cirrhosis was defined by the presence of cirrhosis without the presence of HCV, HBV, or alcoholic liver disease.

The diagnosis of liver fluke infestation was made on the basis of detection of ova or worms in feces, or radiologic finding of diffuse, uniform dilatation of the small intrahepatic bile ducts with no or minimal dilatation of larger bile ducts and with no focal obstructing lesion. Choledochal cysts were considered to be present if there was a characteristic cystic or fusiform dilatation of the extrahepatic or intrahepatic duct on radiologic findings. Choledocholithiasis was defined as the presence of at least one stone in the extrahepatic bile duct, whereas hepatolithiasis as the presence of stone in the intrahepatic bile duct. The presence of cystic duct stone was classified as cholecystolithiasis.

Diabetes was diagnosed according to the World Health Organization Criteria[16], and categorized into two groups: (1) complicated diabetes (presence of any stage of retinopathy, nephropathy or macrovascular complications); and (2) uncomplicated diabetes. Thyroid disease included hyperthyroidism and hypothyroidism.

Statistical analysis
Statistical analyses were performed by using SPSS 20.0 (SPSS incorporated, Chicago, IL, United States). The Mann-Whitney U test and the Pearson χ^2 with Fisher exact test were used to compare continuous and discrete variables, respectively. Univariate and multivariate analyses of correlation were carried out by using conditional logistic regression with maximum likelihood estimates of parameter values for assessing the risk for CCA. Among all variables investigated, primary sclerosing cholangitis, choledochal cyst, and nonspecific liver cirrhosis were not tested because cases were too few to be analyzed ($n < 3$ in whole study population including controls). All other variables were evaluated in the univariable conditional logistic regression analysis, and the variables with $P < 0.1$ in the univariate analysis were included in the multivariable models. The adjusted odds ratio (AOR) and 95%CI for each variable were estimated by using the logistic regression coefficient. In all analyses, $P < 0.05$ for two-sided tests was considered statistically significant.

The synergisms between risk factors were evaluated by including them in the additive regression model using an interaction term, since it is more appropriate to assess biological interactions and public health concerns. Multiple logistic regression models were used to evaluate departure from additivity. By crossing two independent risk factors for CCA, dummy variables of 4 categories were obtained; 2 for the presence of each risk factor alone, 1 for the presence of both risk factors, and 1 for the absence of both risk factors. The last of these categories was used as the reference category in the regression models. To assess the deviation from the additive model of no interaction between variables, the Synergism index (S) and its 95%CI, as proposed by Rothman, was calculated[17]; $S = (OR_{11} - 1)/(OR_{01} + OR_{10} - 2)$. OR_{10} and OR_{01} mean the OR for the presence of each risk factor in the absence of the other, whereas OR_{11} means the OR of the joint effect of two risk factors. A value of S equal to unity was interpreted as indicative of additivity, whereas a value greater than unity was indicative of superadditivity and synergism.

RESULTS
Patient characteristics
There were 276 patients with CCA eligible for this study. Out of these, 83 (30.1%) were ICC and 193 (69.9%) were ECC. The CCA patients and controls had a similar mean age (67.8 ± 12.5 *vs* 67.5 ± 12.5, $P = 0.818$) and proportion of men (50.4% *vs* 50.4%, male to female ratio, 1.02:1), suggesting that pairing was effective.

CCA population
The multivariate conditional logistic analysis showed that cholecystolithiasis (AOR = 1.74; 95%CI: 1.04-2.90), choledocholithiasis (AOR = 12.35; 95%CI: 4.31-35.38),

Table 1 Comparison of risk factors in patients with cholangiocarcinoma and matched controls   *n* (%)

Variable	CCA patient (*n* = 276)	Control (*n* = 552)	Univariable analysis		Multivariable analysis	
			OR (95%CI)	*P* value	AOR (95%CI)	*P* value
Cigarette smoking	84 (30.4)	157 (28.4)	1.14 (0.79-1.66)	0.487	-	-
< 20 pack-years[1]	35 (12.7)	53 (9.6)	1.39 (0.85-2.27)	0.191	-	-
≥ 20 pack-years	49 (17.8)	104 (18.8)	1.01 (0.66-1.55)	0.972	-	-
Heavy alcohol consumption[2]	35 (12.7)	50 (9.1)	1.53 (0.94-2.51)	0.088	1.45 (0.82-2.55)	0.199
Obesity[3]	64 (23.2)	134 (24.3)	0.94 (0.66-1.33)	0.722	-	-
Cholecystolithiasis	47 (17.0)	42 (7.6)	2.34 (1.52-3.61)	< 0.001	1.74 (1.04-2.90)	0.035
Choledocholithiasis	34 (12.3)	7 (1.3)	13.31 (5.20-34.07)	< 0.001	12.35 (4.31-35.38)	< 0.001
Hepatolithiasis	20 (7.2)	1 (0.2)	20.00 (4.68-85.57)	< 0.001	39.87 (7.25-219.17)	< 0.001
Cholecystectomy	17 (6.2)	23 (4.2)	1.49 (0.79-2.82)	0.216	-	-
Ulcerative colitis	2 (0.7)	3 (0.5)	1.33 (0.22-7.98)	0.753	-	-
Alcoholic liver disease	14 (5.1)	26 (4.7)	1.08 (0.55-2.13)	0.816	-	-
Thyroid disease	6 (2.2)	22 (4.0)	0.51 (0.20-1.31)	0.164	-	-
Chronic pancreatitis	1 (0.4)	5 (0.9)	0.40 (0.05-3.42)	0.403	-	-
Hypertension	113 (40.9)	254 (46.0)	0.80 (0.59-1.08)	0.150	-	-
Diabetes mellitus	65 (23.6)	69 (12.5)	2.22 (1.51-3.28)	< 0.001	2.55 (1.66-3.91)	< 0.001
Without complications	36 (13.0)	47 (8.5)	1.82 (1.12-2.96)	0.015	2.20 (1.30-3.70)	0.003
With complications[4]	29 (10.5)	22 (4.0)	2.98 (1.67-5.32)	< 0.001	3.25 (1.69-6.25)	< 0.001
HBV infection	28 (10.1)	18 (3.3)	3.34 (1.80-6.19)	< 0.001	4.12 (2.01-8.44)	< 0.001
HCV infection	11 (4.0)	13 (2.4)	1.69 (0.76-3.78)	0.199	-	-
Liver fluke infestation	6 (2.2)	4 (0.7)	3.00 (0.85-10.63)	0.089	3.49 (0.86-14.07)	0.079

[1]One pack-year = 1 pack per day for a year; [2]Daily amount of ≥ 80 g (male) or ≥ 40 g (female); [3]Obesity was defined as a body mass index > 25 kg/m^2 according to the Asian-Pacific criteria for obesity; [4]Any stage of retinopathy, nephropathy or macrovascular complications. CCA: Cholangiocarcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AOR: Adjusted odds ratio.

hepatolithiasis (AOR = 39.87; 95%CI: 7.25-219.17), HBV infection (AOR = 4.12; 95%CI: 2.01-8.44), and DM (AOR = 2.55; 95%CI: 1.66-3.91) were the significant risk factors for CCA (Table 1). HCV infection and heavy alcohol consumption were not significantly associated with development of CCA. When DM was dichotomized into complicated and uncomplicated DM, complicated DM resulted in a greater risk of CCA than uncomplicated DM (AOR = 3.25 and 2.20, respectively) (Table 1). However, there is no significant correlation between estimated AOR and duration of DM (AOR = 1.42 and 0.75 for 5-10 years and > 10 years, respectively, *P* = 0.5).

Subgroup analysis- ECC and ICC population
Subgroup analysis was performed to investigate risk factors for ECC and ICC development. We included 193 ECC patients and 386 controls, and 83 ICC patients and 166 controls in the conditional logistic regression model. When ECC and ICC cases were compared to their respective control participants, cholecystolithiasis, choledocholithiasis, hepatolithiasis, and DM were the significant risk factors for ECC (AOR = 2.01, 18.08, 14.87 and 1.99, respectively) (Table 2), whereas choledocholithiasis, hepatolithiasis, HBV infection, and DM were the significant risk factors for ICC development (AOR = 8.63, 157.37, 5.27, and 4.87, respectively) (Table 3). Cholecystolithiasis was the significant risk factor for ECC but not ICC development. However, DM was significantly associated with both ECC and ICC. As with the results in the entire CCA population, complicated DM also resulted in a greater risk of CCA than uncomplicated DM in both subgroup analyses, although AOR for uncomplicated DM did not reach statistical significance in

the ECC population (*P* = 0.055) (Tables 2 and 3).

Interaction between risk factors
After evaluating the independent effects of each significant risk factor on CCA development, the inter-actions and synergism of those factors were investigated. Of all significant factors, hepatolithiasis was not included in this analysis because of the small number of cases and lack of controls, with hepatolithiasis and other significant risk factors together. Every pair of other significant risk factors was analyzed with adjustment for the rest of the significant factors. When investigating interactions between diabetes and HBV infection on the risk of CCA, the relative excess risk of developing CCA in patients having DM and HBV infection together exceeded the sum of the relative excess risks for each risk factor alone: 12.2-1.0 > (2.5-1.0) + (3.5-1.0). The estimated synergism index (S) was 2.80 (95%CI: 1.54-5.08), indicating the joint effect of DM and HBV infection is superadditive (Table 4, Figure 1A). When including only complicated diabetes instead of the entire diabetic cases in this analysis, the synergistic effect on the risk of CCA was greater than the effect between DM and HBV infection. The estimated synergism index (S) was 8.12 (95%CI: 4.92-13.38) (Table 4). These superadditivities of the joint effect between DM and HBV infection, or complicated DM and HBV infection were also investigated in the ICC subgroup population. The estimated synergism index (S) between DM and HBV infection, and complicated DM and HBV infection was 2.44 and 5.45, respectively (Table 5, Figure 1B). However, there was no synergistic interaction between other significant risk factors for CCA and ICC. Similarly, no significant interaction was observed between

Table 2 Comparison of risk factors in patients with extrahepatic cholangiocarcinoma and matched controls *n* (%)

Variable	ECC patient (*n* = 193)	Control (*n* = 386)	Univariable analysis		Multivariable analysis	
			OR (95%CI)	*P* value	AOR (95%CI)	*P* value
Cigarette smoking	53 (27.5)	91 (23.6)	1.32 (0.83-2.10)	0.240	-	-
< 20 pack-years	20 (10.4)	30 (7.8)	1.51 (0.79-2.87)	0.212	-	-
≥ 20 pack-years	33 (17.1)	61 (15.8)	1.23 (0.73-2.08)	0.444	-	-
Heavy alcohol consumption	18 (9.3)	24 (6.2)	1.62 (0.83-3.17)	0.161	-	-
Obesity	42 (21.8)	90 (23.3)	0.91 (0.59-1.40)	0.658	-	-
Cholecystolithiasis	33 (17.1)	28 (7.3)	2.49 (1.48-4.20)	0.001	2.01 (1.12-3.58)	0.019
Choledocholithiasis	24 (12.4)	2 (0.5)	24.00 (5.67-101.55)	< 0.001	18.08 (4.18-78.19)	< 0.001
Hepatolithiasis	9 (4.7)	1 (0.3)	18.00 (2.28-142.08)	0.006	14.87 (1.79-123.74)	0.013
Cholecystectomy	13 (6.7)	19 (4.9)	1.38 (0.67-2.84)	0.376	-	-
Ulcerative colitis	2 (1.0)	1 (0.3)	4.00 (0.36-44.11)	0.258	-	-
Alcoholic liver disease	7 (3.6)	13 (3.4)	1.08 (0.42-2.78)	0.871	-	-
Thyroid disease	5 (2.6)	15 (3.9)	0.64 (0.22-1.84)	0.408	-	-
Chronic pancreatitis	1 (0.5)	5 (1.3)	0.40 (0.05-3.42)	0.403	-	-
Hypertension	84 (43.5)	185 (47.9)	0.83 (0.58-1.19)	0.301	-	-
Diabetes mellitus	44 (22.8)	54 (14.0)	1.88 (1.19-2.98)	0.007	1.99 (1.22-3.27)	0.006
Without complications	27 (14.0)	38 (9.8)	1.64 (0.95-2.85)	0.077	1.78 (0.99-3.19)	0.055
With complications	17 (8.8)	16 (4.1)	2.43 (1.18-5.00)	0.016	2.48 (1.16-5.32)	0.020
HBV infection	9 (4.7)	9 (2.3)	2.10 (0.80-5.49)	0.131	-	-
HCV infection	6 (3.1)	10 (2.6)	1.20 (0.44-3.30)	0.724	-	-
Liver fluke infestation	3 (1.6)	2 (0.5)	3.00 (0.50-17.95)	0.229	-	-

HBV: Hepatitis B virus; HCV: Hepatitis C virus; ECC: Extrahepatic cholangiocarcinoma.

Table 3 Comparison of risk factors in patients with intrahepatic cholangiocarcinoma and matched controls *n* (%)

Variable	ICC patient (*n* = 83)	Control (*n* = 166)	Univariable analysis		Multivariable analysis	
			OR (95%CI)	*P* value	AOR (95%CI)	*P* value
Cigarette smoking	31 (37.3)	66 (39.8)	0.87 (0.47-1.63)	0.670	-	-
< 20 pack-years	15 (18.1)	23 (13.9)	1.17 (0.54-2.51)	0.689	-	-
≥ 20 pack-years	16 (19.3)	43 (25.9)	0.68 (0.33-1.44)	0.318	-	-
Heavy alcohol consumption	17 (20.5)	26 (15.7)	1.44 (0.70-2.97)	0.319	-	-
Obesity	22 (26.5)	44 (26.5)	1.00 (0.56-1.80)	0.999	-	-
Cholecystolithiasis	14 (16.9)	14 (8.4)	2.06 (0.96-4.41)	0.062	1.04 (0.33-3.29)	0.941
Choledocholithiasis	10 (12.0)	4 (2.4)	6.20 (1.70-22.71)	0.006	8.63 (1.30-57.33)	0.026
Hepatolithiasis	11 (13.3)	1 (0.6)	22.00 (2.84-170.40)	0.003	157.37 (9.36-2646)	< 0.001
Cholecystectomy	4 (4.8)	4 (2.4)	2.00 (0.50-8.00)	0.327	-	-
Ulcerative colitis	0 (0.0)	2 (1.2)	0.03 (0.0-5748.1)	0.561	-	-
Alcoholic liver disease	7 (8.4)	13 (7.8)	1.09 (0.41-2.87)	0.868	-	-
Thyroid disease	1 (1.2)	7 (4.2)	0.25 (0.03-2.19)	0.211	-	-
Chronic pancreatitis	0 (0.0)	0 (0.0)	-	-	-	-
Hypertension	29 (34.9)	69 (41.6)	0.73 (0.41-1.31)	0.291	-	-
Diabetes mellitus	21 (25.3)	15 (9.0)	3.34 (1.60-7.01)	0.001	4.87 (1.88-12.59)	0.001
Without complications	9 (10.8)	9 (5.4)	2.52 (0.90-7.03)	0.078	4.00 (1.18-13.61)	0.027
With complications	12 (14.5)	6 (3.6)	4.28 (1.59-11.49)	0.004	6.13 (1.57-24.00)	0.009
HBV infection	19 (22.9)	9 (5.4)	4.58 (2.00-10.50)	< 0.001	5.27 (1.93-14.38)	0.001
HCV infection	5 (6.0)	3 (1.8)	3.33 (0.80-13.95)	0.099	1.71 (0.25-11.45)	0.582
Liver fluke infestation	3 (3.6)	2 (1.2)	3.00 (0.50-17.95)	0.229	-	-

HBV: Hepatitis B virus; HCV: Hepatitis C virus; ICC: Intrahepatic cholangiocarcinoma.

significant risk factors for ECC. Only one ECC patient had DM and HBV infection, simultaneously.

DISCUSSION

The etiology and carcinogenesis of CCA remains obscure despite several established risk factors. In the present hospital based case-control study in Korea, we confirmed that HBV infection and DM were independent risk factors for CCA, particularly for ICC development, and found that there was a synergistic interaction between these factors regarding the risk of CCA. In the multivariate conditional logistic regression analysis, choledocholithiasis, hepatolithiasis, and DM were significantly associated with both ECC and ICC, whereas HBV infection and cholecystolithiasis were risk factors only for ICC and ECC development, respectively.

Our investigation of positive association between HBV infection and ICC was consistent with previous reports[7,18]. Previous studies demonstrated that both

Table 4	Interaction between diabetes mellitus and hepatitis B virus infection for cholangiocarcinoma: logistic regression analysis with adjusted odds ratio					
Interaction variables		*n*	β **Coefficient ($\pm$ SE)**	*P* **value**	**AOR[1] (95%CI)**	**S (95%CI)[2]**
DM	HBV					
Negative	Negative	658			1	
Positive	Negative	124	0.909 (0.22)	< 0.001	2.5 (1.6-3.8)	
Negative	Positive	36	1.259 (0.39)	0.001	3.5 (1.6-7.6)	
Positive	Positive	10	2.502 (0.96)	0.009	12.2 (1.9-80.1)	2.80 (1.54-5.08)
Complicated DM	HBV					
Negative	Negative	740			1	
Positive	Negative	42	0.967 (0.34)	0.005	2.6 (1.3-5.1)	
Negative	Positive	37	1.107 (0.38)	0.004	3.0 (1.4-6.4)	
Positive	Positive	9	3.423 (1.32)	0.009	30.7 (2.3-403.4)	8.12 (4.92-13.38)

[1]AOR: Odds ratio adjusted for the other significant risk factors for cholangiocarcinoma; [2]S = Synergy index described by Rothman = (OR$_{11}$ - 1)/(OR$_{01}$ + OR$_{10}$ - 2), where OR$_{11}$ = OR of the joint effect of 2 risk factors; OR$_{01}$ and OR$_{10}$ = OR of each risk factor in the absence of the other. DM: Diabetes mellitus; HBV: Hepatitis B virus; AOR: Adjusted odds ratio.
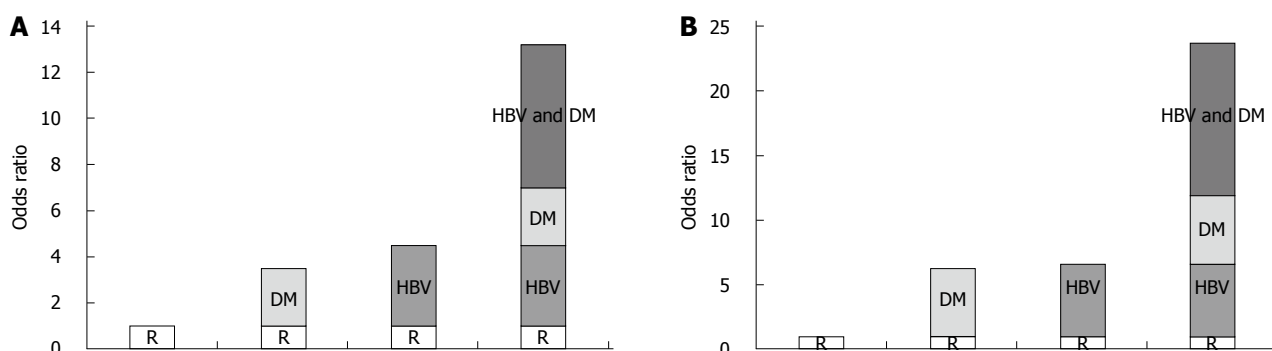

Figure 1 Risk of cholangiocarcinoma in subjects with diabetes mellitus, hepatitis B virus infection, or both. A: Whole cholangiocarcinoma population; B: Intrahepatic cholangiocarcinoma population. DM: Diabetes mellitus; HBV: Hepatitis B virus infection; R: Common reference category.

hepatocytes and cholangiocytes differentiate from the same hepatic progenitor cells; therefore, it is possible that HBV induces carcinogenesis in cholangiocytes through a similar mechanism as in hepatocytes[19,20]. In addition, HBV may be involved in the pathogenesis of ICC through a chronic inflammatory process[21,22]. Chronic inflammation of the biliary epithelium evoked by HBV infection can render it vulnerable to immunologic attack, leading to genetic alterations and subsequent malignant transformations of cells[23].

In contrast with HBV infection, HCV infection was not a significant risk factor for ICC in this investigation. This finding is in accordance with a previous Korean study[7]. Similarly, a recent meta-analysis did not identify a significant association between ICC development and HCV infection when analyzed in relation to East-Asian populations, whereas it did indicate a strong association (OR = 6.91) in relation to Western populations[18]. However, considering the small number of studies and participants analyzed, an additional large-scale study of Eastern regions is warranted to confirm the geographic variation.

Our results also indicated significant association between DM and CCA development, which is compatible with previous studies[7,18]. Insulin resistance and hyperinsulinemia have been shown to stimulate the growth of numerous cancer cell lines[24]. In addition, upregulated insulin-like growth factor 1 may stimulate liver cell proliferation, consequently leading to carcinogenesis of CCA[25,26].

For a more precise assessment, analyses were repeated after subclassifying all diabetic cases according to duration and the presence of complications. Although the duration of DM was not significantly correlated with the CCA risk, the impact of DM on the risk of CCA was greater when DM complications were present. Considering long time interval between actual DM onset and its clinical diagnosis[27], disease duration from diagnosis does not reflect the exact duration of illness. Moreover, control of DM and severity of the disease may be more crucial predictors than mere disease duration. Notably, occurrence of DM complications depends on glucose control and actual disease duration[28]. To clearly elucidate the impact of the duration of DM on the risk of CCA development, a future well-designed study with more detailed information is needed.

In this study, there were several interesting findings regarding the association between cholelithiasis and CCA: (1) cholecystolithiasis was a risk factor for ECC, but not ICC; (2) choledocholithiasis was a risk factor for both ECC and ICC; and (3) hepatolithiasis was a risk factor not only for ICC, but also for ECC. These results

Table 5 Interaction between diabetes mellitus and hepatitis B virus infection for intrahepatic cholangiocarcinoma: logistic regression analysis with adjusted odds ratio

Interaction variables		*n*	*β* Coefficient (± SE)	*P* value	AOR[1] (95%CI)	S (95%CI)
DM	HBV					
Negative	Negative	194			1	
Positive	Negative	27	1.670 (0.51)	0.001	5.3 (2.0-14.3)	
Negative	Positive	19	1.718 (0.58)	0.003	5.6 (1.8-17.4)	
Positive	Positive	9	3.120 (1.15)	0.006	22.7 (2.4-214.1)	2.44 (1.30-4.58)
Complicated DM	HBV					
Negative	Negative	211			1	
Positive	Negative	10	1.660 (0.77)	0.031	5.3 (1.2-23.8)	
Negative	Positive	20	1.528 (0.56)	0.006	4.6 (1.5-13.7)	
Positive	Positive	8	3.782 (1.51)	0.012	43.9 (2.3-849.5)	5.45 (3.16-9.42)

[1]AOR: Odds ratio adjusted for the other significant risk factors for intrahepatic cholangiocarcinoma. DM: Diabetes mellitus; HBV: Hepatitis B virus; AOR: Adjusted odds ratio; S: Synergism index.

may be explained by the effects of cholestasis, altered bile composition, and chronic proliferative inflammation near the stone-bearing ducts. Among the 9 patients with ECC who had hepatolithiasis in our study population, 7 patients had hilar cholangiocarcinoma, which supports this explanation. Previous Chinese studies showed the association between hepatolithiasis and ECC as well[29,30], which also supports our results.

The most noteworthy finding of this study is the synergistic effect between DM and HBV infection on the risk of CCA development. Although the definite mechanism is uncertain, there is a possible explanation for the interaction. Hyperglycemia could stimulate glucose oxidation, lipid peroxidation, and glycosylation of proteins, which leads to production of free radicals causing oxidative stress[31]. Oxidative stress subsequently may promote HBV gene expression, reactivation of viral replication, and liver disease chronicity, leading to DNA damage and CCA development[32,33]. Considering that oxidative stress is a widely accepted key mediator in the progression of DM and its complications[31,34], our finding of greater synergism between complicated DM and HBV infection support this explanation. It is considered that CCA and HCC share common etiologic factors, and a previous study on the risk factors for HCC also indicated the synergistic interaction between DM and HBV infection on cancer development[35,36], supporting our finding as well.

The present study has several potential limitations: (1) diagnostic bias cannot be excluded because cancer patients undergo additional testing, and thus may have more diagnoses than individuals without cancer; (2) this was a hospital-based study performed in a single institution, not a population-based design, therefore there is a possibility of selection bias caused by differential referral patterns; however, hospital-based design may be more appropriate for CCA in view of the low incidence and short survival of CCA patients; (3) hepatitis B core antibody (anti-HBc) and occult HBV infection were not investigated in this study; however, recent studies demonstrated very low levels of HBV DNA in subjects with anti-HBc alone (without surface antigen/antibody), and extremely low incidence of occult HBV infection[37,38]. Furthermore, the prevalence of HBV observed in the present study was comparable with the previous HBV prevalence estimates in Korea[39]; and (4) DM was not classified according to type; considering the absence of young-onset (≤ 30 years) DM in our cohort and extremely lower incidence of type I DM in Asia[40], most of the DM cases in this study were thought to be type 2 DM.

Despite these limitations, our study has several noteworthy strengths: (1) this is the first analysis of risk factors for ECC in Korea that was conducted after adjustment for possible confounding risk factors in a multivariable model. A previous Korean study[41] focused only on *Clonorchis sinensis* parasitosis as a risk factor for ECC without adjustment for confounders; (2) the prevalence of the significant risk factors in the control subjects was comparable with the prevalence in the general population of Korea[42,43] or other Asian country[44]; (3) we stratified biliary lithiasis and DM according to the location and the presence of the complication, respectively. To the best of our knowledge, this is the first study to investigate the impact of complicated and uncomplicated DM on CCA risk, after stratification of DM; and (4) most importantly, this is the first study to investigate the synergistic effect between DM and HBV infection on the risk of CCA development. This "new" finding may help stratify patients at risk for CCA and design CCA surveillance algorithms depending on the stratification.

In conclusion, besides the biliary lithiasis, HBV infection and DM were independent risk factors for CCA, especially for ICC development. In addition, there was synergistic interaction between the two factors on the risk for CCA development. A further large-scale study is warranted to confirm this synergistic interaction and to clarify possible underlying mechanisms. Exploring the underlying mechanisms for such synergy may lead to the development of CCA prevention strategies in high-risk individuals.

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COMMENTS

Background

Previous studies have identified several risk factors for cholangiocarcinoma (CCA) development. However, the etiology of CCA is still largely unknown. Moreover, the interaction between risk factors has not been investigated to date.

Research frontiers

Liver fluke infestation and hepatolithiasis were established as risk factors for CCA. In the recent investigations, hepatitis B virus (HBV) infection and diabetes mellitus (DM) were reported as significant risk factors for CCA as well.

Innovations and breakthroughs

In this study, the authors found that the impact of DM on the risk of CCA was greater when diabetic complications were present. The results showed that there was a synergistic effect between HBV infection and DM on the risk of CCA, and that the synergistic effect was enhanced in cases of complicated DM.

Applications

The authors' findings may help stratify patients at risk for CCA and design CCA surveillance algorithms depending on such stratification. Exploring the underlying mechanisms for synergy between HBV infection and DM may lead to the development of CCA prevention strategies in high-risk individuals.

Peer review

This is a well-designed and relevant study, showing that HBV infection and DM exert independent and synergistic effects on the risk for CCA, including intrahepatic CCA. The methodology is well described, and the statistics are sound. It makes a significant contribution to our understanding of the CCA etiology.

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