

RAPID COMMUNICATION

Risk factors for intrahepatic cholangiocarcinoma: A case-control study in China

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Abstract

AIM: To carry out a hospital-based case-control study to investigate risk factors for intrahepatic cholangiocarcinoma (ICC) in China.

METHODS: A total of 312 ICC cases and 438 matched controls were included in the study. The presence of diabetes mellitus, hypertension, hepatolithiasis, primary sclerosing cholangitis, liver fluke infection (*Clonorchis sinensis*), was investigated through clinical records. Blood from all participants was tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using conditional logistic regression.

RESULTS: Compared with controls, ICC patients had a higher prevalence of HBsAg seropositivity (48.4% vs 9.6%, $P < 0.000$), and hepatolithiasis (5.4% vs 1.1%, $P = 0.001$). By multivariate analysis, the significant risk factors for development of ICC were HBsAg seropositivity (adjusted OR, 8.876, 95% CI, 5.973-13.192), and hepatolithiasis (adjusted OR, 5.765, 95% CI, 1.972-16.851). The prevalence of anti-HCV seropositivity, diabetes mellitus, hypertension, cigarette smoking, and alcohol consumption were not significantly different between cases and controls.

CONCLUSION: These findings suggest that HBV infection and hepatolithiasis are strong risk factors for development of ICC in China.

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Key words: Intrahepatic cholangiocarcinoma; Hepatitis B virus; Hepatitis C virus; Risk factors

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC), a rare malignant tumor arising from the ductular epithelium of the biliary tree within the liver, is the second most common malignancy after hepatocellular carcinoma (HCC) among the primary liver cancers^[1]. It is one of the most biologically virulent malignancies and has a dismal prognosis because of its late diagnosis and absence of effective treatment. There is a marked regional variation in the incidence of ICC, linked strongly to the distribution of risk factors^[2]. Therefore, identifying risk factors may lead to prevention and control of this disease. Liver fluke infection (*Clonorchis sinensis* or *Opisthorchi viverrini*)^[3,4], primary sclerosing cholangitis (PSC)^[5,6], and hepatolithiasis are thought to be the risk factors for ICC^[7,8]. Recently, several studies have suggested that HBV and HCV infections, the major risk factors for the development of HCC, also increase the risk of ICC^[9-13]. However, to the best of our knowledge, no study of ICC and risk factors has yet been conducted in China.

We therefore conducted a hospital-based case-control study to investigate risk factors for ICC in China, a country endemic for HBV infection.

MATERIALS AND METHODS

Subjects

This study consisted of 312 patients with ICC who underwent surgical resection at the Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China, between February 2004 and May 2006. Controls were selected from patients who were unaffected by liver diseases in the Changhai Hospital of the Second

Table 1 Characteristics of cases and controls

Characteristics	ICC (n = 312)	Control (n = 438)	P value
Mean age (SD)	53.2 ± 10.4	54.8 ± 11.8	> 0.05
Gender			> 0.05
Male	207 (66%)	276 (63%)	
Female	105 (34%)	162 (37%)	

Military Medical University, and were matched to cases in terms of age (± 5 years), sex, and date of hospital admission. The control group consisted of 438 patients admitted to the Departments of Gastroenterology, Urology, Ophthalmology, Neurology and Cardiology. All the specimens from ICC were diagnosed by pathological examination.

For both cases and controls, ever having smoked cigarettes was defined as having smoked cigarettes ≥ 4 d/wk for ≥ 6 mo, and ever having drunk alcohol as having consumed alcoholic beverages ≥ 1 d/wk for ≥ 6 mo.

The presence of diabetes mellitus, hypertension, hepatolithiasis, PSC, liver fluke infection (*C. sinensis*), was investigated through clinical records.

Laboratory tests

Blood samples were taken from all patients on the first morning of hospital admission, and tested for HBsAg and anti-HCV using a commercial ELISA (Abbott Laboratories, North Chicago, IL, USA). The project was approved by the ethics committee at the two hospitals.

Statistical analysis

Categorical variables were compared using the χ^2 test and continuous variables were compared using Student's *t* test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using a conditional multiple logistic regression model. The model was constructed using a forward selection process. $P < 0.05$ was considered statistically significant. These analyses were performed using SPSS 11.0 software (SPSS, Chicago, IL, USA).

RESULTS

No differences were found between patients with ICC and controls according to age and gender (Table 1). Table 2 shows the results of univariate analysis on the risk factors for ICC. The prevalence of anti-HCV seropositivity, diabetes mellitus, hypertension, cigarette smoking and alcohol consumption were not significantly different between the patient and control groups. Compared with the controls, ICC patients had a higher prevalence of HBsAg seropositivity (48.4% *vs* 9.6%, $P < 0.000$), and hepatolithiasis (5.4% *vs* 1.1%, $P = 0.001$). None of the patients had PSC. Liver fluke infection was found in 11 ICC cases (3.5%). However, it is difficult to obtain accurate diagnostic evidence for liver fluke infection and PSC in control groups. Thus, we could not compare these two variables between the patients and controls.

All variables in the univariate analysis were further evaluated by multivariate logistic regression analysis. The

Table 2 Risk factors for ICC by multivariate logistic regression analysis

Variables	ICC Cases n = 312 (%)	Controls n = 438 (%)	OR (95% CI)
HBV			8.876 (5.973-13.192)
HBsAg+	151 (48.4)	42 (9.6)	
HBsAg-	161 (51.6)	396 (90.4)	
HCV			0.933 (0.281-3.1)
Anti-HCV+	9 (2.9)	6 (1.4)	
Anti-HCV-	303 (97.1)	432 (98.6)	
Diabetes mellitus			1.505 (0.597- 3.796)
Yes	13 (4.2)	11 (2.5)	
No	299 (95.8)	427 (97.5)	
Hypertension			1.492 (0.867-2.570)
Yes	34 (10.9)	38 (8.7)	
No	278 (89.1)	400 (91.3)	
Hepatolithiasis			5.765 (1.972-16.851)
Yes	17 (5.4)	5 (1.1)	
No	295 (94.5)	433 (98.9)	
Cigarette smoking			1.237 (0.7-2.188)
Yes	43 (13.8)	67 (15.3)	
No	269 (86.2)	371 (84.7)	
Alcohol consumption			0.809 (0.487-1.345)
Yes	39 (12.5)	41 (9.4)	
No	273 (87.5)	397 (90.6)	

significant independent risk factors for development of ICC were HBsAg seropositivity (adjusted OR, 8.876, 95% CI, 5.973-13.192) and hepatolithiasis (adjusted OR, 5.765, 95% CI, 1.972-16.851). Other variables were not significant risk factors by multivariate logistic regression analysis.

The mean age was significantly younger in the HBsAg-positive ICC group (50.8 \pm 10.4 years) compared with the HBsAg-negative group (55.5 \pm 9.9 years). There were more men among HBsAg-positive ICC patients compared with the HBsAg-negative group [76.8% (116/151) *vs* 56.5% (91/161); $P < 0.001$]. Among ICC patients, cirrhosis was found in 69 who were HBsAg-positive (45.7%), and in six who were HBsAg-negative (3.7%).

DISCUSSION

Recently, infection with HCV or HBV has been suggested as a risk factor for ICC^[3,9-13]. A case-control (41 cases and 406 controls) study from Korea reported that the prevalence of anti-HCV and HBsAg positivity was 12.5% and 13.8%, respectively, in patients compared with 3.5% and 2.3% in controls. Multivariate analysis found anti-HCV positivity to be associated with ICC (OR, 3.9) but not with HBsAg positivity (OR, 1.3)^[3]. A prospective cohort study from Japan showed that 2.3% of 600 patients with HCV-related cirrhosis developed ICC during a mean follow-up of 7.2 years. The risk of developing ICC among patients with HCV-related cirrhosis was 1000 times higher than that in the general population^[9]. A case-control study from Italy that compared 26 patients with ICC with 824 controls found that seropositivity for anti-HCV and HBsAg was 25 and 13% in ICC cases and 5.8 and 6.7% in controls, respectively. A statistically significant increase in the OR was observed for anti-HCV (OR, 9.7), whereas a weaker, non-significant association was found with HBsAg (OR, 2.7)^[10]. Similarly, according to a case-control study

that involved 625 cases and 90 834 controls from the United States, the prevalence of anti-HCV and HBsAg was 5.6 and 0.2% in ICC cases and 1 and 0.2% in controls, respectively. The adjusted OR for ICC was 6.1 for anti-HCV, whereas no association was found with HBsAg (OR, 0.8)^[11]. Another hospital-based case-control study from Japan showed that HCV seropositivity was detected in 36% of ICC patients and 3% of controls, and the adjusted OR for ICC development was 6.02 for anti-HCV^[12]. Overall, these studies suggest that HCV infection may play a role in ICC development, whereas HBV infection does not seem to be a relevant risk factor for the disease. Only sporadic cases of ICC have been reported with HBV infection^[13]. In the current study, seropositivity for HBsAg and anti-HCV were found in 48.4 and 2.9% of ICC cases, and in 9.6 and 1.4% of controls, respectively. This indicates that HBV, but not HCV infection is a strong risk factor for development of ICC. These findings differed markedly from the results of previous studies^[3,9-12]. ICC is characterized by wide variability in incidence and risk factors. HCV seems to be associated with ICC in regions with relatively low prevalence of HBV infection, such as Japan and the United States^[9,11]. China, a highly endemic area for HBV infection, has an HBsAg-positive rate of 9.09% in the general population^[14], but a low incidence of HCV infection (0.42%-2.1%)^[15,16]. Therefore, the difference in etiological factors associated with ICC can be explained by the type of endemic hepatitis virus. Thus, screening and vaccination for hepatitis B may be a useful strategy to decrease the high rate of ICC in China. It is noteworthy that the prevalence of HBV or HCV infection in our controls is compatible with the figure observed in the general population in China, which suggests no relevant selection bias in our controls.

The precise mechanism of HBV infection in the carcinogenesis of ICC is still unknown. Integration of the HBV sequence into the human genome is one of the most important steps in HBV-related carcinogenesis^[17]. HBV sequences are found in human^[18] and duck^[19] bile duct epithelial cells by *in situ* hybridization. HBV sequence integration is suggested to have a direct oncogenic effect by modulating the expression of neighboring cellular genes involved in the regulation of cell proliferation and apoptosis. HBV X gene-encoded protein (HBX) acts as a transactivator of various cellular genes and plays a crucial role in the pathogenesis of HCC^[17,20]. Wang *et al*^[21] has observed frequent, strong HBX immunohistochemical staining in bile duct cells of cancerous and surrounding hepatic tissues, in some HBV-infected ICC specimens, and has suggested that HBX may contribute to the pathogenesis of ICC. Furthermore, it has been shown that HBX may activate the transcriptional expression of human telomerase reverse transcriptase, which leads to tumorigenesis in cholangiocytes^[22]. Further studies are required to investigate the cellular transformation mechanisms of ICC induced by HBV infection.

Cirrhosis, such as alcoholic cirrhosis and HCV-related cirrhosis, has also been associated with ICC^[9,10]. HBV infection may result in chronic hepatitis, followed by liver cirrhosis. Our data showed that cirrhosis was found in 69

HBsAg-positive ICC cases (45.7%). This finding suggests that HBV-related cirrhosis may be an essential stage in the development of ICC, as it is for HCC in China.

Hepatolithiasis was another independent risk factor for ICC development in our study. This result was compatible with several previous studies^[17,8,23,24]. The carcinogenesis associated with hepatolithiasis may be a multi-step process in which there are changes from hyperplasia to dysplasia, and finally to adenocarcinoma^[25]. Recent studies have demonstrated that several cancer-related genes, including those of *p16*, cyclooxygenase-2, prostaglandin E2, c-met, and caudal-related homeobox gene 2, play an important role in the development and progression of ICC arising in hepatolithiasis^[26-30].

In conclusion, this study showed that HBV, but not HCV infection and hepatolithiasis, are strong risk factors for development of ICC in China.

COMMENTS

Background

Intrahepatic cholangiocarcinoma (ICC) is one of the most biologically virulent malignancies and has a dismal prognosis because of its late diagnosis and absence of effective treatment. There is a marked regional variation in the incidence of ICC, linked strongly to the distribution of risk factors^[2]. Therefore, identifying risk factors may lead to prevention and control of this disease. HBV and HCV infections have been suggested as risk factors for ICC^[3,9-13]. However, no study of ICC and risk factors has yet been conducted in the China, a country endemic for HBV infection.

Research frontiers

We carried out a hospital-based case-control study to investigate risk factors for ICC in China. A total of 312 ICC cases and 438 matched controls were included in the study.

Innovations and breakthroughs

We found that HBV, but not HCV infection and hepatolithiasis were strong risk factors for development of ICC in China.

Applications

Screening and vaccination for hepatitis B may be a useful strategy to decrease the high rate of ICC in China.

Peer review

This is a well performed study that shows that HBV, but not HCV infection and hepatolithiasis are strong risk factors for development of ICC in China. The discussion is well balanced.

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