Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma

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Although hepatitis C virus (HCV)-related cirrhosis has been suggested as a risk factor for intrahepatic cholangiocarcinoma (ICC), few sizeable studies have tested this hypothesis. We investigated ICC risk factors, with special reference to HCV infection. We conducted a hospital-based case-control study including 50 ICC patients and 205 other surgical patients without primary liver cancer. HCV seropositivity was detected in 36% of ICC patients and 3% of controls. By univariate analysis, the odds ratio (OR) for association of anti-HCV antibodies with development was 16.87 (95% confidence interval (CI), 5.69 to 50.00). History of blood transfusion or diabetes mellitus, elevated serum total bilirubin, elevated aspartate aminotransferase and alanine aminotransferase, decreased serum albumin and decreased platelet count were identified as other possible ICC risk factors. By multivariate analysis, anti-HCV antibodies (adjusted OR, 6.02; 95% CI, 1.51 to 24.1), elevated alanine aminotransferase, decreased serum albumin, and decreased platelet count were found to be independent risk factors for ICC development. As liver status worsened, the adjusted OR for ICC tended to increase. HCV infection is a likely etiology of ICC in Japan. (Cancer Sci 2004; 95: 592–595)

Intrahepatic cholangiocarcinoma (ICC), a malignant tumor arising from bile duct epithelium, is the second most common primary liver cancer, following hepatocellular carcinoma (HCC). Established risk factors for HCC include chronic active hepatitis, and hepatic fibrosis induced by hepatitis virus infection or heavy alcohol intake. On the other hand, risk factors for ICC remain uncertain. Hepatitis C virus (HCV)-related cirrhosis has been suggested as a risk factor for ICC,[1, 2] but confirmation is required. We therefore investigated risk factors for ICC, with special reference to HCV infection.

Patients and Methods

We conducted a hospital-based study using a matched case-control design, including 50 patients with pathologically diagnosed ICC and 205 other surgical patients without primary liver cancer including ICC.

Patients. Fifty patients were treated for ICC between January 1991 and December 2002 in the two major medical centers of Osaka City (Department of Gastroenterological and Hepato-Biliary-Pancreatic Surgery, Osaka City University Hospital; Department of Gastrointestinal Surgery, Osaka City General Hospital). ICC was diagnosed by pathologic examination. Two to five control patients at the two medical centers were matched with each ICC patient according to gender, 5-year age group, and operation date (within 1 year). Patients with HCC were excluded from the control group since hepatitis B virus (HBV) infection and HCV infection are established etiologic agents of HCC. In all, control patients numbered 205. This study was conducted in accordance with the Helsinki Declaration and the guidelines of the ethics committees at our institutions.

Etiologic factors and data collection. Factors evaluated for association with ICC risk included habits such as alcohol consumption and smoking; family history of liver disease; past history of blood transfusion (before 1990); other medical conditions such as cholelithiasis including hepatolithiasis, diabetes mellitus, and hypertension; liver status (chronic hepatitis and liver cirrhosis); and results of laboratory tests such as anti-HCV antibody, HB surface antigen, serum concentrations of albumin and total bilirubin, serum activities of aspartate aminotransferase and alanine aminotransferase, and platelet count. Blood was collected at the time of the first medical examination; the separated serum was stored at −80°C until the time of assay. Positivity for anti-HCV antibody and HB surface antigen was determined with only these sera. Medical records of cases and controls were reviewed with respect to habits, family history, past history, other medical conditions, and liver status at operation. Alcohol consumption described in medical records was converted into *sake* (Japanese wine) equivalents. One "*go*" (180 ml) of *sake* contains about 27 ml of ethanol and is equal in alcohol content to 60 ml of whisky, 240 ml of wine, or 633 ml of beer. Cases and controls were divided into three groups (light, moderate, or heavy drinkers) based on their alcohol consumption and frequency of drinking. A heavy drinker was defined as drinking the equivalent of at least 5 *go* of per day for more than 10 years. Moderate drinkers consumed from at least 3 *go* to 5 *go* of *sake* per day for 10 years. Light drinkers consumed less than 3 *go* of *sake* per day. Subjects were considered smokers if they had smoked for any time within the 5 years before admission. Diabetes mellitus and hypertension were defined in terms of requirements for appropriate oral medications or injections.

Blood samples were tested for HB surface antigen and anti-HCV antibody using enzyme-linked immunosorbent assay kits (International Reagent, Kobe, Japan). Laboratory test results were classified as either within or outside the reference range. As for liver status, patients were classified as having a normal liver, chronic hepatitis, or cirrhosis, based on laboratory test results or pathologic examination of a hepatic tissue specimen.

Statistical analyses. For univariate analysis, the relationship between each possible risk factor and development of ICC was analyzed with the χ^2 test, Fisher's exact test, or the Mantel extension test as appropriate. Then univariate analysis of correlation was carried out using conditional logistic regression with maximum likelihood estimates of parameter values for assessing the risk of ICC. Odds ratio (OR) and 95% confidence interval (CI) were calculated using a conditional logistic regression model. For multivariate analysis, variables showing significance ($P<0.1$) in the univariate analysis were modeled using conditional logistic regression. The adjusted OR and 95% CI for each variable were estimated using the logistic regression coefficient. These analyses were performed using SAS version 8.12; Statistical Analysis System, United States.

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Results

Patients' characteristics. Distributions of gender, age, and associated diseases in ICC control groups are shown in Tables 1 and 2. Surgical diseases present in controls were classified as benign or malignant (Table 2). Benign diseases included digestive, endocrine, respiratory, and cardiovascular disorders. Malignant diseases included digestive, mammary, endocrine, and respiratory cancers. Of 50 ICC patients, 4 patients had simultaneous HCC in another part of the liver. In another patient, ICC was detected 8 years after resection of the primary HCC. All 5 patients with both ICC and HCC were infected with HCV.

Risk factors for ICC development.

Univariate analysis: Percentages of anti-HCV antibody seropositivity were 36% in ICC patients and 3% in controls (Table 3). Patients with anti-HCV antibodies had an OR for ICC development of 16.87 (95% CI, 5.69 to 50.00). Presence of HBsAg, family history of liver disease, alcohol consumption, smoking, and other medical conditions such as hypertension or cholelithiasis were not significantly associated with ICC development. The OR for ICC development with a history of blood transfusion was 3.14 (1.26 to 7.83), and that with diabetes mellitus was 2.19 (0.98 to 4.90); thus, these are possible risk factors. On the other hand, hypertension tended to be more common among controls than ICC patients (OR, 0.50; 0.23 to 1.11). ORs with chronic hepatitis and cirrhosis were 4.70 (2.18 to 10.10) and 18.72 (3.43 to 102.05), respectively. An elevated serum concentration of total bilirubin (OR, 3.85; 1.88 to 7.88), elevated activities of aspartate aminotransferase (OR, 9.95; 4.67 to 21.21) and alanine aminotransferase (OR, 5.26; 2.62 to 10.58), a decreased serum concentration of albumin (OR, 3.56; 1.72 to 7.35), and a low platelet count (OR, 3.18; 1.61 to 6.28) were significantly associated with ICC development.

Multivariate analysis: Except for aspartate aminotransferase activity, possible risk factors according to univariate analysis were entered in the multivariate analysis. By multivariate analysis, anti-HCV antibody seropositivity (adjusted OR, 6.02; 1.51 to 24.1), elevated alanine aminotransferase activity (adjusted OR, 2.89; 1.13 to 7.38), a low serum albumin concentration (adjusted OR, 2.85; 1.04 to 7.79), and a low platelet count (adjusted OR, 2.39; 0.96 to 5.95) were independent risk factors for ICC development. As liver status worsened, the adjusted OR for ICC tended to increase (chronic hepatitis, 2.32 vs. cirrhosis, 5.03; $P<0.09$).

Table 1. Characteristics of cases and matched controls

		Cases	Controls
Gender: number (%)	Male	29 (58%)	121 (59%)
	Female	21 (42%)	84 (41%)
Age: mean±SD		64.6±9.7	65.3±9.2

Gender and age distributions were very similar in cases and controls.

Discussion

Several factors have been reported as etiologic factors of ICC. Liver fluke infection (*Clonorchis sinensis* or *Opisthorchis viverrini*),[3] primary sclerosing cholangitis, and hepatolithiasis[4] have been reported to be etiologic factors in ICC.[1] Recently infection with HCV or HBV has been suggested to be involved in the pathogenesis of ICC,[1, 2, 5, 6] although some investigators consider development of ICC to be unrelated to viral hepatitis.[7, 8] Few epidemiologic studies have been undertaken to settle this issue because of the small numbers of patients with ICC. In this study, 36% of ICC patients were infected with HCV. In contrast, the percentage of control patients infected with HCV was 3%, which is compatible with the HCV infection rate (1.5% and 3%) in the general population of Osaka.[9–11] Our study indicated that HCV is a strong risk factor for development of ICC. A large study should be performed in a bigger population as a health examination survey, because our study was conducted in only two medical centers. A national surveillance study by the Liver Cancer Study Group of Japan demonstrated that the prevalence of HCV infection in ICC patients between 1990 and 1997 was 28.3% in men and 26.6% in women,[3] significantly higher than the national seropositivity rate of approximately 1.5%.[9] The prevalence of HCV infection determined among ICC patients in our hospitals was also higher than the percentages in the national surveillance study, which may reflect the specific region studied; Osaka has the

Table 2. Diseases in controls

Benign (75)		Malignant (130)	
Digestive (20)	Cholelithiasis (10)	Digestive (60)	Gastric cancer (15)
	Esophageal hiatus hernia (2)		Esophageal cancer (22)
	Gallbladder polyp (2)		Colon cancer (13)
	Ventral hernia (2)		Pancreas cancer (8)
	Rectal polyps (1)		Small intestinal tumor (1)
	Hepatolithiasis (1)		Pelvic tumor (1)
	Ileus (1)		
	Lipoma (1)		
Mammary and endocrine (2)	Adenomatous goiter (2)	Mammary and endocrine (26)	Breast cancer (18)
			Thyroid cancer (8)
Respiratory (2)	Thymoma (1)	Respiratory (44)	Lung cancer (39)
	Mediastinitis (1)		Pleural tumor (2)
			Mediastinal tumor (2)
			MALT of lung (1)
Cardiovascular (51)	Angina pectoris (20)		
	Valvular disorder (15)		
	Aneurysm (12)		
	Atrial septal defect (1)		
	Aortic dissection (1)		
	Superior vena cave syndrome (1)		
	Arteriosclerosis obliterans (1)		

Patients were divided into two major groups representing, benign and malignant disease. MALT: mucosa associated lymphonodi tumor.

Table 3. Statistical analyses

Predictive factors		ICC patients (n=50)		Controls (n=205)		Univariate	Multivariate	
		n	%	n	%	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)	P value
HCV	anti-HCV (–)	32	64	198	97	1	1	
	anti-HCV (+)	18	36	7	3	16.87 (5.69–50.00)	6.02 (1.51–24.1)	0.01
HBV	HBsAg (–)	48	96	200	98	1		
	HBsAg (+)	2	4	5	2	1.84 (0.34–10.11)		
Family history	(–)	47	94	189	92	1		
	(+)	3	6	16	8	0.75 (0.22–2.61)		
Smoking	(–)	33	66	115	56	1		
	(+)	17	34	90	44	0.65 (0.32–1.32)		
Transfusion	(–)	41	82	191	93	1	1	
	(+)	9	18	14	7	3.14 (1.26–7.83)	0.76 (0.22–2.70)	0.68
Diabetes mellitus	(–)	39	78	181	88	1	1	
	(+)	11	22	24	12	2.19 (0.98–4.90)	1.95 (0.65–5.85)	0.23
Hypertension	(–)	40	80	141	69	1	1	
	(+)	10	20	64	31	0.50 (0.23–1.11)	0.46 (0.16–1.35)	0.16
Cholelithiasis	(–)	47	94	181	88	1		
	(+)	3	6	24	12	0.41 (0.12–1.48)		
Liver status (at operation)	Normal liver	30	60	183	90	1	1	
	Chronic hepatitis	15	30	19	9	4.70 (2.18–10.10)	2.32 (0.70–7.64)	0.17
	Cirrhosis	5	10	2	1	18.72 (3.43–102.05)	5.03 (0.45–56.82)	0.19
								Trend: P<0.09
Alcohol consumption	Mild or moderate	49	98	94	95	1		
	Heavy	1	2	11	5	0.97 (0.49–1.89)		
T-Bil (mg/dl)	<0.1	28	56	167	81	1	1	
	≥0.1	22	44	38	19	3.85 (1.88–7.88)	1.71 (0.67–4.37)	0.26
AST (IU/liter)	<40	19	38	177	86	1		
	≥40	31	62	28	14	9.95 (4.67–21.21)		
ALT (IU/liter)	<40	27	54	178	87	1	1	
	≥40	23	46	27	13	5.26 (2.62–10.58)	2.89 (1.13–7.38)	0.03
Alb (g/dl)	≥3.5	32	64	175	85	1	1	
	<3.5	18	36	30	15	3.56 (1.72–7.35)	2.85 (1.04–7.79)	0.04
Plt ($\times 10^4/\mu l$)	≥18	30	60	167	82	1	1	
	<18	20	40	36	18	3.18 (1.61–6.28)	2.39 (0.96–5.95)	0.06

Factors entered in the multivariate analysis were those that showed a significant difference in ICC risk by univariate analysis. ICC, intrahepatic cholangiocarcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B virus surface antigen; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alb, albumin; Plt, platelet.

highest prevalence of HCV infection in Japan.[10, 11] Indeed, the prevalence of HCV infection among ICC patients differs considerably among areas.[8, 12] Where HCV infection is prevalent, it is an important pathogenetic factor in ICC. Although a high prevalence of HBV infection in ICC patients has been reported by the Liver Cancer Study Group of Japan (4.0% to 14.5%),[13] we did not find HBV infection to be an ICC risk factor in this study.

The high OR associated with blood transfusion before 1990 is likely to reflect HCV infection via transfusion. Although a relationship between alcohol consumption and ICC was found by some investigators,[7, 8] alcohol consumption was not a risk factor for development of ICC in this study. Cirrhosis has been reported to be a risk factor for ICC.[2, 3, 7, 8, 12, 14] In essential agreement, we found worsening of liver fibrosis status (via chronic hepatitis to cirrhosis) to be associated with development of ICC. By univariate analysis, an elevated serum concentration of total bilirubin, elevated activities of aspartate aminotransferase and alanine aminotransferase, a decreased serum concentration of albumin, and a decreased platelet count were significant risk factors for ICC. By multivariate analysis, elevated alanine aminotransferase, a decreased serum albumin, and a decreased platelet count remained as independent ICC risk factors. These factors are related to HCV infection and progression of hepatic fibrosis. Thus, impaired liver function as a result of HCV infection and tissue reactions provoked by it are a risk factor for ICC.

Exactly how ICC might develop in patients infected with HCV still is unclear, but various mechanisms have been proposed. HCV core antigen has been identified in proliferating bile ductules in chronic hepatitis C,[15] and infection of these structures might be associated with the development of ICC.[1] On the other hand, in relation to the hypothesis of a combined HCC-ICC pathogenetic sequence,[16, 17] cells morphologically intermediate between hepatocytes and bile duct epithelium have been noted during the process of carcinogenesis. When predominantly bile duct cells develop from these proliferating common precursors, ICC would result. Interestingly, 5 of the 50 ICC patients in this study also developed HCC either simultaneously or at a different time. A similar finding has also been reported by Kobayashi *et al.*[2]

Although curative surgical resection provides the only chance of long-term survival for patients with ICC, their postoperative prognosis presently is poor because many patients are diagnosed with ICC at an advanced stage.[15] This unfortunate situation may be changing. Yamamoto *et al.*[1] reported 12 patients who underwent liver resection for an ICC smaller than 3 cm; in 10 of these patients the tumor was detected during follow up for chronic hepatitis C. Thus, close follow-up of patients infected by HCV should result in a higher rate of early detection of ICC, as well as HCC.

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