locus were associated with two serum markers of inflammation: TNF-α and soluble intercellular adhesion molecule 1 (sICAM-1) [14,15]. Enhanced expression of TNF-α is associated with liver inflammation and hepatocarcinogenesis[16], also plasma levels of sICAM-1 are not only associated with the risk of incident diabetes and liver disease activity, known predisposing factors for HCC, but furthermore are also a predictive marker of HCC occurrence and prognosis [17,18,19,20,21,22]. These indicate a possible link between the ABO blood group and HCC risk, especially in the presence of other etiological risks for HCC. We hypothesized that certain serotypes of the ABO blood group might provide additional risk of HCC development in the presence of CHB. So, the aim of this case-control study was to examine the association between the ABO blood group and HCC risk among patients with CHB.

Materials and Methods

Study population

This case-control study was part of an ongoing hospital-based prospective investigation [23] that was conducted in Jinan Infectious Disease Hospital, Shandong University, a tertiary hospital in Shandong Province, China. The study was approved by the ethics committee of Jinan Infectious Disease Hospital, Shandong University, and written informed consent for participation was obtained from each study participant.

Subjects who fulfilled the following criteria were recruited into the study: hospitalized for HCC or CHB, age ≥30 years, positive for hepatitis B surface antigen (HBsAg), negative for anti-HCV, without a history or other evidence of cancer other than HCC, without a history or other evidence of hepatitis other than hepatitis B, without a history of alcohol consumption, no cancer treatment, and no treatment with nucleotide/nucleosides or interferon, Han population and residence of Shandong Province.

From January 2004 and December 2008, a total of 6,275 consecutive eligible patients (1,105 HBV-related HCC and 5170 CHB without HCC) were enrolled. 882 eligible patients were not recruited because of patient refusal or patient sickness. Statistical analyses indicated that the eligible patients who were not recruited did not differ from the recruited patients in term of demographic and clinical features (retrieved from patients' medical records).

A total of 1105 HBV-related HCC patients were used as the case in the study. The 5170 hospital cross-sectional CHB patients without HCC were used as the control.

Study variables

The variables analyzed in this study included age, sex, city of residence, family history of liver cancer, type 2 diabetes, hepatitis B e antigen (HBeAg), cirrhosis, HBV DNA level, and the ABO blood group. Biochemical parameters related to impaired liver function, such as, bilirubin, albumin, and prothrombin time, were also analyzed to explore the relationship between the ABO blood group and severity of liver disease.

Upon entry to the hospital, all subjects were interviewed by trained physicians. Demographic data, family histories, and medical histories were collected. Physical examinations, blood counts, the ABO blood type, Rh factor, serum biochemical parameters, prothrombin time, serum alpha-fetoprotein (AFP), anti-HCV (AxSYM HCV, version 3.0, Abbott Laboratories, Abbott Park, IL, USA), hepatitis B surface antigen (HBsAg), anti-HBs, HBeAg, anti-HBe, and anti-HBc (Abbott Laboratories) were all measured. HBV DNA quantification (Roche COBAS HBV Amplicor Monitor assay), ultrasound examinations, and gastrointestinal barium meal X-ray examinations were also performed.

First-degree relatives (parents, siblings, and children) with liver cancer were considered to have a positive family history of liver cancer. Subjects with a fasting blood sugar level of at least 7.0 mmol/L on at least two occasions or those diagnosed with type 2 diabetes before entering the hospital were defined as having type 2 diabetes. At the hospital, a standardized questionnaire was used to interview the patients about their history of alcohol drinking, including drinking frequency, average monthly intake of each type of alcoholic beverage. We assessed total alcohol intake according to the average ethanol content, by volume, of beer (4–5 percent), wine (grape wine, rice wine 8–12 percent), and liquor(38–60 percent)[24]. Subjects with an ethanol intake of 30 g/d or more for men and 20 g/d or more for women for longer than 10 years were considered to have a positive history of alcohol consumption. A structured questionnaire was used to record the information collected.

Diagnosis of HCC and Cirrhosis

The criteria for diagnosis of HCC were positive histology or cytology, two image findings showing HCC from different sources [ultrasonography, enhanced computed tomography (CT) scan, or magnetic resonance imaging(MRI)], or serum AFP level greater than 400 ng/mL in combination with one positive image finding. Image diagnosis for HCC was based on the following classic imaging manifestations: early enhancement at the arterial phase and hypoattenuation at the portal venous phase or at the equilibrium phase on contrast-enhanced dynamic CT or MRI, and hyperattenuation on CT during hepatic arteriography and hypoattenuation on CT during arterial portography. Of these 1,105 cases, 298 cases were diagnosed by histological pathology, 364 cases with an enhanced computed tomography scan and magnetic resonance imaging, and 443 cases with a finding of AFP higher than 400 ng/mL in combination with an abnormal finding from an enhanced computed tomography scan or ultrasonography.

Diagnosis of cirrhosis was based on liver biopsy or clinical findings combined with at least one image finding from ultrasonography or a computed tomography scan. In 386 CHB patients with a biopsy, the diagnosis of cirrhosis was based on liver histology according to the criteria of Desmet et al. [25]. In those patients without biopsy specimens, the diagnosis of cirrhosis was based on clinical and morphological criteria, ultrasound or computed tomography, according to standard definitions[26]. These included the presence of clinical manifestations of portal hypertension (e.g., esophageal varices, encephalopathy, or ascites), biochemical abnormalities (e.g., decreased serum albumin and platelets or prolonged prothrombin time), and obvious morphological changes in the liver detected by hepatic imaging (e.g., ultrasonography or computed tomography scan). Minor signs were also clinically noted, such as palmar erythema, spider angioma, and clubbing of the fingers. Ultrasonography was performed by experienced radiologists for every patient upon entry to the hospital. The severity of cirrhosis was evaluated using Child-Pugh score[27].

Statistical Analysis

Demographic and clinical parameters were evaluated using the chi-squared test for categorical variables, independent-Samples t-test for continuous variables with normal distribution, and the Mann-Whitney U test or Kruskal-Wallis test for continuous variables with skewed distribution. Possible confounding effects among the variables were adjusted using a multivariate logistic regression model, and adjusted odds ratios (AORs) and 95% confidence intervals (CI) were calculated. Effect modifications

Table 1. Demographic and clinical characteristics of hepatitis B virus-related HCC cases and hospital cross-sectional CHB controls: Univarite analyses.

	Cases	Controls		
Variables	**N=1105 (%)**	**N=5170 (%)**	**Univariate OR (95%CI)**	***P***
Sex				<0.001
Female	169 (15.3)	1353 (26.2)	1	
Male	936 (84.7)	3817 (73.8)	1.96 (1.65–2.34)	
Age (years)				<0.001
30–39	82 (7.4)	2014 (39.0)	1	
40–49	250 (22.6)	1385 (26.8)	4.43 (3.42–5.74)	
50–59	470 (42.5)	1247 (24.1)	9.26 (7.25–11.83)	
≥60	303 (27.4)	524 (10.1)	14.20 (10.93–18.46)	
City of residence				0.69
Jinan	464 (42.0)	2137 (41.3)	1	
Other cities	641 (58.0)	3033 (58.7)	1.03 (0.90–1.17)	
Family history of liver cancer				0.76
No	1030 (93.2)	4832 (93.5)	1	
Yes	75 (6.8)	338 (6.5)	1.04 (0.80–1.35)	
Type 2 diabetes				0.013
No	1012 (91.6)	4842 (93.7)	1	
Yes	93 (8.4)	328 (6.3)	1.36 (1.07–1.73)	
HBeAg status				<0.001
HBeAg+	256 (23.2)	3144 (60.8)	1	
HBeAg−	849 (76.8)	2026 (39.2)	5.15 (4.43–5.98)	
Cirrhosis				<0.001
No	346 (31.3)	3559 (68.8)	1	
Yes	759 (68.7)	1611 (31.2)	4.85 (4.21–1.73)	
HBVDNA>10^5 copies/mL				<0.001
No	559(50.6)	1153(22.3)	1	
Yes	546(49.4)	4017(77.7)	0.28 (0.24–0.33)	

HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; OR, odds ratio; CI, confidence interval; HBeAg, hepatitis B e antigen.
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were evaluated by stratification, statistical interaction was assessed by including main effect variables and their product terms in the logistic regression model. For all tests, *P* values were 2 sided. A *P* value of less than 0.05 was considered significant. All data analyses were performed using SPSS v. 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient Characteristics

The demographic and clinical characteristics of HCC cases and CHB controls are summarized in **Table 1.** As expected, most HCC patients were male (84.7%), HBeAg negative (76.8%), and cirrhotic (68.7%). The prevalence of HBV DNA>10^5copies/mL were lower among cases than among controls. Cases and controls had a similar distribution of family history of liver cancer and city of residence (Jinan and other cities of Shandong Province). HCC patients were older than controls, the mean age ± standard deviation was 53.8±9.3 years for patients with HCC and 44.9±10.7 years for controls ($P<0.001$).

ABO blood group and HCC risk

Distributions of blood types O, A, AB, and B are shown in **Table 2.** The distribution of blood type A was higher among HCC cases than among CHB controls (29.0% vs 24.9%, $P=0.013$), whereas, the distribution of blood type O, AB, and B was similar between cases and controls. Compared with subjects with blood type O, the unadjusted OR for the association of those with blood type A and HCC risk was 1.31(95% CI, 1.06–1.61). In multivariable logistic regression, the AOR was 1.39(95%CI, 1.05–1.83) after adjusting for age, sex, type 2 diabetes, cirrhosis, HBeAg, and HBV DNA.

We further assessed whether the association between the ABO blood group and HCC risk differed according to strata of other known risk factors for HCC, including sex, HBeAg, HBV DNA level, and cirrhosis. An interaction between ABO blood type and sex or HBeAg was observed. The AOR (95% CI) for the interaction term of blood type A, B, or AB and sex was 1.57 (0.78–3.17), 3.33 (1.60–6.92), and 8.93 (1.91–41.64), respectively. The AOR (95%) for the interaction term of blood type A, B, or AB and HBeAg was 4.82 (2.58–9.02), 2.30 (1.23–4.32), and 2.64 (1.11–6.30), respectively. As shown in **Table 3**, among male patients, compared to subjects with blood type O, those with blood type A, or B were at a greater HCC risk, whereas, among female patients, compared with blood type O, those with blood type AB or B were at a lesser HCC risk. A strong association between the ABO blood

Table 2. ABO blood type and risk for hepatocellular carcinoma development in patients with chronic hepatitis B.

	Cases	Controls			
ABO type	**N=1105 (%)**	**N=5170 (%)**	**Univariate OR (95%CI)**	**AOR (95%CI)**	***P***
O	277 (25.1)	1462 (28.3)	1(reference)	1(reference)	
A	321 (29.0)	1289 (24.9)	1.31 (1.06–1.61)	1.39 (1.05–1.83)	0.021
AB	132 (11.9)	609 (11.8)	1.13 (0.87–1.48)	0.85 (0.58–1.25)	0.409
B	375 (33.9)	1810 (35.0)	1.09 (0.89–1.32)	1.14 (0.87–1.49)	0.337
A+AB	453 (41.0)	1898 (36.7)	1.25 (1.04–1.52)	1.23 (0.95–1.59)	0.122
B+AB	507 (45.9)	2419 (46.8)	1.10 (0.91–1.32)	1.05 (0.81–1.35)	0.732
A+B+AB	828 (74.9)	3708 (71.7)	1.17 (0.99–1.39)	1.18 (0.93–1.49)	0.175

AOR, adjusted odds ratio; CI, confidence interval.
Logistic regression model adjusted for sex, age, type 2 diabetes, cirrhosis, HBeAg status, and HBVDNA.
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group and HCC risk was observed among HBeAg positive patients, but not among HBeAg negative patients.

ABO blood group and severity of liver disease

As shown in **Table 4**, subjects with blood type A had more severely impaired liver function. Compared with those with other blood types, subjects with blood type A had a significantly high prevalence of abnormal bilirubin and prolonged prothrombin time. The distribution of cirrhosis was not significantly higher among subjects with blood type A than among those with other blood types. However, among the age<55 years group, the prevalence of cirrhosis was significantly higher among subjects with blood type A than among those with non-A. This indicated a relationship between blood type A and early onset of cirrhosis.

Discussion

In this large case-control study in Chinese patients with CHB, we observed a significantly elevated risk for HCC development among those having blood type A, compared to those having blood type O. Stratified analysis indicated that the association was significant in men and not significant in women after adjusting

Table 3. ABO blood type and hepatocellular carcinoma risk in patients with chronic hepatitis B: stratified multivariate analyses.

	ABO blood type				
Factors	**O**	**A**	**AB**	**B**	**A+AB+B**
Sex					
Female					
No. of cases/controls	64/395	47/311	7/130	51/517	105/958
AOR(95%CI)	1 (reference)	1.07(0.56–2.06)	0.11(0.02–0.53)	0.45(0.23–0.88)	0.55(0.32–0.93)
***P* value**		0.83	0.005	0.019	0.027
Male					
No. of cases/controls	213/1067	274/978	125/479	324/1293	723/2750
AOR(95%CI)	1(reference)	1.56(1.14–2.13)	1.15(0.76–1.73)	1.41(1.05–1.91)	1.42(1.09–1.86)
***P* value**		0.005	0.511	0.024	0.009
HBeAg status					
HBeAg positive					
No. of cases/controls	47/927	100/708	19/377	90/1132	209/2217
AOR(95%CI)	1(reference)	4.92(2.83–8.57)	1.89(0.87–4.12)	2.25(1.28–3.96)	3.03(1.83–5.03)
***P* value**		<0.001	0.11	0.005	<0.001
HBeAg negative					
No. of cases/controls	230/535	221/581	113/232	285/678	619/1491
AOR(95%CI)	1(reference)	0.81(0.58–1.12)	0.63(0.41–0.99)	0.90(0.66–1.23)	0.82(0.62–1.07)
***P* value**		0.203	0.043	0.519	0.15
Cirrhosis					
No					
No. of cases/controls	100/995	105/866	41/432	100/1266	246/2564
AOR(95%CI)	1(reference)	1.10(0.67–1.79)	0.77(0.40–1.48)	0.85(0.52–1.41)	0.92(0.60–1.40)
***P* value**		0.803	0.442	0.515	0.69
Yes					
No. of cases/controls	177/467	216/423	91/177	275/544	582/1144
AOR(95%CI)	1(reference)	1.57(1.12–2.20)	0.86(0.54–1.38)	1.32(0.96–1.81)	1.32(1.0–1.75)
***P* value**		0.008	0.537	0.090	0.05
HBV DNA>10^5copies/mL					
No					
No. of cases/controls	125/354	143/306	78/136	203/357	424/799
AOR(95%CI)	1(reference)	1.58(1.04–2.42)	1.08(0.63–1.84)	1.68(1.13–2.50)	1.52(1.07–2.17)
***P* value**		0.033	0.776	0.011	0.02
Yes					
No. of cases/controls	152/1108	168/983	54/473	172/1453	394/2909
AOR(95%CI)	1(reference)	1.22(0.85–1.77)	0.71(0.41–1.25)	0.80(0.56–1.16)	0.94(0.68–1.29)
***P* value**		0.282	0.234	0.239	0.69

AOR, adjusted odds ratio; CI, confidence interval; HBeAg, hepatitis B e antigen. Logistic regression model adjusted for sex, age, type 2 diabetes, cirrhosis, HBeAg status, and HBVDNA, and excluding the stratification variable.

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Table 4. ABO Blood type and severity of liver disease in chronic Hepatitis B.

	O	A	AB	B	Non-A		
Variables	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	$P^{\#}$	P^{*}
Bilirubin≥50 μmol/L	314 (28.7)	322 (33.2)	113 (23.9)	327 (23.9)	754 (25.7)	<0.001	<0.001
Albumin<35g/L	317 (28.9)	305 (31.4)	143 (30.2)	365 (26.7)	825 (28.1)	0.125	0.065
Prothrombin≥18.5s	147 (13.4)	157 (16.2)	69 (14.6)	149 (10.9)	365 (12.4)	0.011	0.011
With Cirrhosis	644 (37.0)	639 (39.7)	268 (36.1)	819 (37.5)	1731 (37.1)	0.339	0.082
Age<55years	398 (30.8)	430 (35.2)	166 (29.9)	493 (29.9)	1057 (30.2)	0.032	0.004
Age≥55years	246 (55.3)	209 (53.7)	102 (54.8)	326 (60.5)	674 (57.6)	0.314	0.304

#Chi-Square test among blood type O, A, AB, and B;
*A vs. Non-A.
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confounding factors. Compared with men with blood type O, those with blood type A or B had a significantly high HCC risk that was independent of established HCC risk factors. To the best of our knowledge, this is the first study showing an association between the ABO blood group and HCC risk in patients with CHB. However, a few previous studies showed relationships between the ABO blood group and liver diseases, including HCC [28,29,30,31], so the discovery of a relationship between the ABO blood group and HCC is not surprising.

There are three reasons. First, the ABO blood group was associated with the severity of liver disease, one of the major predicators for HCC[5]. Poujol-Robert et al. reported that non-O blood group is an independent risk factor for the progression of liver fibrosis in HCV infection[28]. We also observed the association between the ABO blood group and severity of liver disease in our study population. Compared to those with blood type O, those with blood type A had a significantly more severely impaired liver function and an earlier onset of cirrhosis. These indicated an association between the ABO blood group and liver inflammation and fibrosis progression in patients with CHB.

The second reason that a relationship between the ABO blood group and HCC is that the ABO blood group in conjunction with several important cytokines known to be related to HCC development, including EGF, TNF-α, sICAM-1, E-selectin, and P-selectin et al [14,15,32,33,34].

The EGF receptor (EGFR) plays a link between inflammation and liver cancer[35], and EGF gene polymorphisms have been reported to be associated with HCC risk[10,12]. The binding of epidermal growth EGF to EGFR is different in blood group A and O, and loss or gain of expression of the A antigen may significantly alter the binding properties of that protein affecting cell signaling and growth[33]. An increase in the number of high affinity EGF binding sites was observed in donors with blood group A1-erythrocytes as compared to red cells taken from donors with blood groups O and B. Glycosyltranferase specificity has broad implications, beyond defining the ABO blood group. Glycoconjugates are important mediators of intercellular adhesion and membrane signaling, two processes integral to malignant progression and spread [36]. The results of two recent GWAS which reported the associations of SNPs at the ABO locus with plasma levels of TNF-α and sICAM-1 also suggested a possible link between the ABO blood group and HCC risk [14,15].

TNF is a pleiotropic cytokine involved not only in apoptosis, but also with inflammation, hepatocyte protection, proliferation and hepatocarcinogenesis [10,37]. Polymorphisms of TNF-α is associated with HCC risk [11].The mechanism of the association between the ABO blood group and TNF-α levels is not known. Potentially, the TNF-α-ABO association is mechanistically related to the association between E-selectin and ABO because it is known that TNF-α induces E-selectin expression and is positively associated with E-selectin levels[34,38,39]. Interestingly, another GWAS reported the ABO is a major locus for serum soluble E-selectin levels[34]. Whether the association between levels of TNF-α and E-selectin can be explained by their respective association with ABO is not clear.

ICAM-1 belongs to the immunoglobulin superfamily and plays an important role in the regulation of immune response, particularly in the antigen-presenting mechanism[40]. Plasma levels of sICAM-1 have been reported to be associated with liver disease activity, HCC occurrence and prognosis [17,20,21,22]. There is growing evidence that the ABO blood group is highly significantly associated with variation in the levels of a number of biomarkers. A very recent large-scale genomic study revealed sP-selectin levels were also associated with ABO gene variants and the association was accounted for by the A1 allele of the ABO blood group [32].

P-selectin, which has important roles in inflammatory processes, tumor formation and metastasis, is a member of the selectin family of adhesion molecules and is expressed mainly at the surface of platelets and endothelial cells [41,42].These indicated a possible association of ABO blood group with platelets. Platelets are key actors in inflammation and critical for liver regeneration[43]. Interestingly, an association between the ABO blood group and thrombocytopenia in patients with CHB was observed (unpublished data). Thrombocytopenia has been regarded as a surrogate of liver fibrosis and a predicator of HCC [44,45]. So, platelets might mediate another possible inflammatory pathway connecting the ABO blood group with liver fibrosis and HCC risk.

The physiological role of the ABO blood group still remains enigmatic. The ABO blood type loci are not in linkage with genes encoding ICAM, E-selectin, and P-selectin. ABO gene product related glycosylation might influence sheding/cleavage of these biomarkers from the endothelium, probably by glycosylation of P-selectin, E-selectin, and ICAM-1[46]. Glycosylation could also affect the clearance rate of sP-selectin, sE-selectin, and SICAM-1 from blood[32,34]. Decreased cleavage of adhesion molecules from endothelial cells associated with A allele would mean more adhesion molecules on the endothelial cells, increased adhesion and inflammation[14]. In conclusion, collective data suggested that the ABO blood type A might increase HCC risk by connecting with several inflammatory pathways.

Third, abnormal expression ABO blood antigens in liver tissue might be another possible explanation for a relationship between the ABO blood group and HCC. ABO blood antigens (A, B, H) usually express on the surface of red blood cells and most epithelial tissue, but not on hepatocytes, sinusoidal endothelial cells, and bile canaliculi of the normal liver[47]. However, an increased ABH expression or neoexpression was observed in HCC tissues. Terada et al. reported that expression of the ABO blood group antigen was more severe in atypical adenomatous hyperplasia and hepatocellular carcinoma than in normal liver and chronic hepatitis[30]. Okada found neoexpression of ABH blood group antigens in HCC tissues[31]. Expression of H-active glycolipid was enhanced in HCC tissues from the patients with blood types other than O. These suggest that alterations in glycosyltransferase specificity may occur during hepatocarcinogenesis. Recently, Hoshida and colleagues provided new insights into genome-based predictors of outcome in HCC patients [48].If abnormal expression of the ABO blood group antigen in liver tissue could predict HCC occurrence and outcome warrants further study.

We observed a gender difference in the association between the ABO blood group and HCC risk, women with blood group AB or B had a significantly lower HCC risk than those with blood group O. Because of the small number of women with blood type AB in the present study, the result should be considered with caution. The underlying mechanism of the gender difference is unknown. However, gender-related HCC risk difference is universal. The overall male to female ratio of HCC is about 2:1 to 4:1[3]. A gender difference was observed in that interleukin-6 signaling promotes chemically induced HCC in genetic mouse models [49]. Several epidemiological studies also showed gender-based dimorphism for the association between other risk factors and HCC development [50,51]. The present data indicated blood type A was an additional HCC risk in the presence of other potent HCC etiological risk factors. However, in women, a low HCC risk population compared with men, the role of blood A antigen on HCC development might be not potent enough to become a significant additional HCC risk factor. Interestingly, another finding that there was no significant association between blood type A and HCC risk among subjects without cirrhosis, a low HCC risk population compared with those with cirrhosis, also supported the notion.

This study has several notable strengths. First its large sample size enabled us to get a meaningful finding of the association between the ABO blood group and HCC risk in patients with CHB. Second, inpatient CHB controls in this study largely represents the population from which the HBV-related HCC cases arose. In areas of high HBV endemicity, persons with cirrhosis have an approximately 3-fold higher risk for HCC than those with chronic hepatitis but without cirrhosis and a 16-fold higher HCC risk than the inactive carrier[5]. So, most HBV-related HCC arose from CHB with or without cirrhosis, but not from an inactive HBsAg carrier. Third, some possible confounding factors, such as cirrhosis status, alcohol consumption, family history of liver cancer, city of residence, HBeAg status, and HBV DNA were evaluated in this study.

There are certain limitations to the study. First, the study population was composed primarily of Chinese Han patients with CHB, which somewhat limits the generalization of the results. Further studies are necessary to confirm the association in other population and in patients with other underlying liver diseases. Second, not all HCC and cirrhosis cases were histologically diagnosed. However, regarding HCC diagnosis, all patients with nodules of 1–2 cm were confirmed with positive cytohistological findings by ultrasound-guided liver biopsy. All of the patients were re-evaluated during the hospitalization period. Moreover, hospitalized HCC patients in this study were followed-up every 1–3 months in the outpatient division of our hospital. Thus, it is believed that there were no cases of misdiagnosed HCC, or, at the very least, there were very few.

In conclusion, the results suggested that ABO blood type is associated with the risk of HCC in Chinese patients with CHB. The ABO blood group A increased HCC risk in patients with CHB independent of other major HCC risk factors, and this association was gender-related. Further studies are necessary to confirm the association in patients with other underlying liver diseases and to elaborate mechanisms by which ABO antigens may influence HCC risk. In the future, it is possible that the ABO blood type could be incorporated into predictive models for HBV-related HCC, together with other human genetic, HBV-related risk, and environmental factors.

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Author Contributions

Conceived and designed the experiments: QL Z-TG S-JC. Performed the experiments: QL J-HY LL S-SX XY W-BF C-HY. Analyzed the data: QL Z-TG S-JC NK. Contributed reagents/materials/analysis tools: QL C-HY J-HY W-WL. Wrote the paper: QL NK.

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Type 2 diabetes and hepatocellular carcinoma: a case–control study in patients with chronic hepatitis B

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Type 2 diabetes has been suggested as an independent risk factor for the development of hepatocellular carcinoma (HCC). However, the role of Type 2 diabetes on the development of HCC in the presence of chronic hepatitis B (CHB) remains inconclusive. We conducted this hospital-based case–control study to evaluate the roles of Type 2 diabetes in HCC development in patients with CHB. From January 2004 to December 2008, a total of 6,275 eligible consecutive patients with chronic hepatitis B virus (HBV) infection were recruited. A total of 1,105 of them were patients with HBV-related HCC and 5,170 patients were CHB but without HCC. We used multivariate logistic regression models to investigate the association between Type 2 diabetes and HCC risk. The prevalence of Type 2 diabetes is higher among HCC patients without cirrhosis than among those with cirrhosis (12.1% *vs.* 6.7%, $p = 0.003$). Type 2 diabetes was associated with a significantly high risk of HCC in female patients after adjusting for age, family history of HCC, city of residence, hepatitis B e antigen and cirrhosis with an odds ratio (95% confidence interval, CI) of 1.9 (1.1–3.4). Restricted analyses among female patients without cirrhosis indicated that Type 2 diabetes was strongly associated with HCC risk with adjusted odds ratio (95% CI) of 5.6 (2.2–14.1). In conclusion, Type 2 diabetes is independently associated with the increased risk of HCC in female CHB patients. Female CHB patients with Type 2 diabetes are of a high HCC risk population and should be considered for HCC close surveillance program.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with 55% occurring in China alone.[1,2] In China, nearly 80% of HCC cases have been linked to hepatitis B virus (HBV) infection and approximately 60–90% of these develop in patients with cirrhosis.[3,4] Other potential risk factors, such as diabetes mellitus, alcohol abuse and obesity, may also play a role in the development of HCC.[5]

Key words: Type 2 diabetes, hepatocellular carcinoma, hepatitis B, gender

Abbreviations: AFP: alpha-fetoprotein; AOR: adjusted odds ratio; CHB: chronic hepatitis B; CI: confidence intervals; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen

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A number of cohort and case–control studies have investigated the relationship between diabetes mellitus and HCC risk.[6–15] Type 2 diabetes has been suggested as an independent risk factor for the development of HCC. However, the role of Type 2 diabetes in the development of HCC in the presence of chronic hepatitis B (CHB) has not been well documented. First, only a few cohort studies have followed a population with chronic HBV infection. In addition, most of the case–control studies used a normal population or cancers other than HCC as controls, and chronic HBV infection status was not well matched between cases and controls.[16] Second, none of these studies followed a cohort of patients with CHB or matched cirrhosis status between cases and controls with CHB. The majority of HBV-related HCC develops in patients with cirrhosis,[17] in which the prevalence of Type 2 diabetes is higher than in the general population, and in CHB patients without cirrhosis.[18] So, these studies may also inappropriately estimate the role of Type 2 diabetes in HCC development in the presence of CHB. Third, whether Type 2 diabetes plays the same role in HCC development between men and women in the presence of CHB is not clear. It has been speculated that in the absence of other strong etiological risk factors for HCC, such as cirrhosis and male gender, Type 2 diabetes plays an additional role on its development.

Thus, we have conducted this hospital-based case–control study to more precisely evaluate the role of Type 2 diabetes

in the development of HCC in the presence of CHB and to explore whether Type 2 diabetes plays the same role in HCC development in males and females and in cirrhotic and non-cirrhotic patients with CHB.

Methods

Study design and population

This case–control study is part of an ongoing hospital-based prospective investigation[19] that was conducted in Jinan Infectious Disease Hospital, a tertiary hospital in Shandong, China. The study was approved by the hospital ethics committee, and written informed consent for participation was obtained from each study participant.

Subjects who fulfilled the following criteria were recruited into the study: hospitalized for HCC or CHB, age $\geq$ 30 years, positive for hepatitis B surface antigen (HBsAg), negative for anti-HCV, without a history or other evidence of cancer other than HCC, without a history or other evidence of hepatitis other than hepatitis B, no cancer treatment, and no treatment with nucleotide/nucleosides or interferon, residence of Shandong Province. From January 2004 and December 2008, a total of 8,027 eligible consecutive patients (1,410 HBV-related HCC and 6,617 CHB without HCC) were enrolled. A total of 882 eligible patients (9.9%) were not recruited because of patient refusal or patient sickness. Statistical analyses indicated that the eligible patients who were not recruited did not differ from the recruited patients in terms of demographic and clinical features (retrieved from patients' medical records). As non-HCC patients with CHB requiring hospital care may be more likely to have heavy alcohol use than HCC patients, 305 HCC cases and 1,447 CHB patients with alcohol consumption were excluded from subsequent analysis.

A total of 1,105 HBV-related HCC patients were used as the case. The 5,170 hospital cross-sectional CHB patients without HCC were used as the control.

Study variables

The variables analyzed in our study included age, sex, city of residence, family history of liver cancer, hepatitis B e antigen (HBeAg), cirrhosis, platelet count, Child-Pugh grade and Type 2 diabetes.

On entry to the hospital, all subjects were interviewed in person by trained physicians. Demographic data, family histories and medical histories were collected. Physical examinations, blood counts, serum biochemical parameters, prothrombin time, serum alpha-fetoprotein (AFP), anti-HCV (AxSYM HCV, version 3.0, Abbott Laboratories, Abbott Park, IL), HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc (Abbott Laboratories) were all measured. HBV DNA quantification (Roche COBAS HBV Amplicor Monitor assay), ultrasound examinations and gastrointestinal barium meal X-ray examinations were also performed. First-degree relatives (parents, siblings and children) with liver cancer were considered to have a positive family history of liver cancer. A structured questionnaire was used to record the information collected.

At the hospital, a standardized questionnaire was used to interview the patients about their history of alcohol drinking, including drinking frequency, average monthly intake of each type of alcoholic beverage. We assessed total alcohol intake according to the average ethanol content, by volume, of beer (4–5%), wine (grape wine, rice wine 8–12%) and liquor (38–60%).[20] Subjects with an ethanol intake of 30 g/day or more for men and 20 g/day or more for women for longer than 10 years were considered to have a positive history of alcohol consumption.

Diagnosis of HCC and cirrhosis

The criteria for diagnosis of HCC were positive histology or cytology, two imaging findings showing HCC from different sources (ultrasonography, enhanced computed tomography scan or magnetic resonance imaging), or serum AFP level greater than 400 ng/mL in combination with one positive image finding. Of these 1,105 cases, 298 cases were diagnosed by histological pathology, 364 cases with an enhanced computed tomography scan and magnetic resonance imaging and 443 cases with a finding of AFP higher than 400 ng/mL in combination with an abnormal finding from an enhanced computed tomography scan or ultrasonography.

Liver cirrhosis was histologically or clinically diagnosed. In patients with a biopsy, the diagnosis of cirrhosis was based on liver histology according to the criteria of Desmet *et al.*[21] In patients without biopsy specimens, the diagnosis of cirrhosis was based on clinical and morphological criteria, ultrasound or computed tomography, according to standard definitions.[22] These included the presence of clinical manifestations of portal hypertension (*e.g.*, esophageal varices, encephalopathy or ascites), biochemical abnormalities (*e.g.*, decreased serum albumin and platelets or prolonged prothrombin time) and obvious morphologic changes in the liver detected by hepatic imaging. Minor signs were also noted clinically, such as palmar erythema, spider angioma and clubbing of the fingers. Ultrasonography was performed by experienced radiologists for every patient on entry to the hospital.[23] The severity of cirrhosis was evaluated using Child-Pugh score.[24]

Diagnosis of Type 2 diabetes

Diagnosis of diabetes mellitus was based on the 1999 World Health Organization criteria.[25] Subjects who were found to have a fasting blood sugar level between 5.6 and 6.1 mmol/L were defined as having impaired fasting glucose and were referred for confirmation of their diabetes status. Those with a fasting blood sugar level of at least 7.0 mmol/L on at least two occasions or those diagnosed with Type 2 diabetes before entering the hospital were defined as having Type 2 diabetes and were referred for further diabetic care. For patients with a history of diabetes mellitus, the type of diabetes, the age at diagnosis and the duration of diabetes were also recorded.

Table 1. Demographic and clinical characteristics of hepatitis B virus-related HCC cases and hospital cross-sectional CHB controls

	All		Women		Men	
Variables	Cases, $N = 1,105$ (%)	Controls, $N = 5,170$ (%)	Cases, $N = 169$ (%)	Controls, $N = 1,353$ (%)	Cases, $N = 936$ (%)	Controls, $N = 3,817$ (%)
Sex						
Female	169 (15.3)	1,353 (26.2)				
Male	936 (84.7)	3,817 (73.8)				
Age (years)						
30–39	82 (7.4)	2,014 (39.0)	10 (5.9)	393 (29.0)	72 (7.7)	1,621 (42.5)
40–49	250 (22.6)	1,385 (26.8)	30 (17.8)	393 (29.0)	220 (23.5)	992 (26.0)
50–59	470 (42.5)	1,247 (24.1)	63 (37.3)	363 (26.8)	407 (43.5)	884 (23.2)
≥60	303 (27.4)	524 (10.1)	66 (39.0)	204 (15.1)	237 (25.3)	320 (8.4)
City of residence						
Jinan	464 (42.0)	2,137 (41.3)	73 (43.1)	637 (47.1)	391 (41.8)	1,500 (39.3)
Other cities	641 (58.0)	3,033 (58.7)	96 (56.8)	716 (52.9)	545 (58.2)	2,317 (60.7)
Family history of liver cancer						
No	1,030 (93.2)	4,832 (93.5)	153 (90.5)	1,268 (93.7)	877 (93.7)	3,564 (93.4)
Yes	75 (6.8)	338 (6.5)	16 (9.5)	85 (6.3)	59 (6.3)	253 (6.6)
Type 2 diabetes						
No	1,012 (91.6)	4,842 (93.7)	149 (88.2)	1,281 (94.7)	863 (91.6)	2,561 (93.7)
Yes	93 (8.4)	328 (6.3)	20 (11.8)	72 (5.3)	73 (7.8)	256 (6.7)
HBeAg status						
HBeAg+	256 (23.2)	3,144 (60.8)	32 (18.9)	827 (61.1)	224 (23.2)	2,317 (60.8)
HBeAg–	849 (76.8)	2,026 (39.2)	137 (81.1)	526 (38.9)	712 (76.8)	1,500 (39.2)
Platelet $< 150 \times 10^9$/L						
No	308 (28.9)	1,852 (35.3)	40 (28.9)	478 (35.3)	268 (28.6)	1,374 (36.0)
Yes	797 (71.1)	3,318 (64.7)	129 (71.1)	875 (64.7)	668 (71.4)	2,443 (64.0)
Cirrhosis						
No	346 (31.3)	3,559 (68.8)	54 (32.0)	895 (66.1)	292 (31.2)	2,664 (69.8)
Yes	759 (68.7)	1,611 (31.2)	115 (68.0)	458 (33.9)	644 (68.8)	1,153 (30.2)
Child-pugh grade						
A	174 (22.9)	365 (22.7)	29 (25.2)	120 (26.2)	145 (22.5)	245 (21.2)
B	345 (45.5)	543 (33.7)	55 (47.8)	138 (30.1)	290 (45.0)	405 (35.1)
C	240 (31.6)	703 (43.6)	31 (27.0)	200 (43.7)	209 (32.5)	503 (43.6)

Abbreviations: HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen.

Patients with prior diagnosis of Type 1 diabetes were excluded from the study.

Statistical analysis

Clinical parameters were evaluated using the chi-squared test for categorical variables and the Mann–Whitney U test or Kruskal–Wallis test for continuous variables. Possible confounding effects among the variables were adjusted using a multivariate logistic regression model, and adjusted odds ratios (AORs) and 95% confidence intervals (CI) were calculated. Effect modifications were evaluated by stratification, statistical interaction was assessed by including main effect variables and their product terms in the logistic regression model. Considering platelet count and Child-Pugh grade which reflect the severity of liver disease have been reported to be associated with HCC risk,[12,26] these two variables were also adjusted, respectively, in the logistic regression models stratified by cirrhosis. For all tests, a p value of less than 0.05 was considered significant. All data analyses were performed using SPSS v. 13.0 (SPSS, Chicago, IL).

Results

Patient characteristics

The demographic and clinical characteristics of HCC patients and their hospital cross-sectional CHB controls are summarized in Table 1. Most HCC patients were male (84.7%),

HBeAg negative (76.8%) and cirrhotic (68.7%). Cases and controls had a similar distribution of family history of liver cancer and city of residence (Jinan and other cities of Shandong Province). HCC patients were older than controls; the mean age ± standard error was 53.8 ± 9.3 for patients with HCC and 44.9 ± 10.7 for controls. The mean age ± standard error of male and female subjects with HCC was 53.4 ± 9.1 years and 56.0 ± 10.0 years ($p = 0.001$), respectively. The mean age of HCC patients with cirrhosis and without cirrhosis was similar (53.9 ± 9.0 *vs.* 53.6 ± 9.8, $p = 0.54$). After adjusting age, sex, city of residence, family history of liver cancer, HBeAg status and cirrhosis, multiple logistic regression analyses indicate the AOR (95% CI) of HCC risk for male sex, old age (10-year increment) and cirrhosis are 2.6 (2.2–3.2), 2.0 (1.8–2.1) and 2.8 (2.4–3.3), respectively.

Table 2. Prevalence of Type 2 diabetes in different age, sex and cirrhosis status groups and duration of diabetes among hepatitis B virus-related HCC cases and hospital cross-sectional CHB controls

Variables	Overall, *N* (%)	Cases, *N* (%)	Controls, *N* (%)	*p* Value*
Age (years)				
30–39	50 (2.4)	1 (1.2)	49 (2.4)	0.72
40–49	110 (6.7)	18 (7.2)	92 (6.6)	0.78
50–59	183 (10.7)	39 (8.3)	144 (11.5)	0.05
≥60	78 (9.4)	35 (11.6)	43 (8.2)	0.14
Sex				
Female	92 (6.0)	20 (11.8)	72 (5.3)	0.003
Male	329 (6.9)	73 (7.8)	256 (6.7)	0.25
Cirrhosis				
No	230 (5.9)	42 (12.1)	188 (5.3)	<0.001
Yes	191 (8.1)	51 (6.7)	140 (8.7)	0.10
Duration of diabetes >5 years	97 (23.0)	34 (36.6)	63 (19.2)	<0.001

*HCC *vs.* CHB.
Abbreviations: HCC, hepatocellular carcinoma; CHB, chronic hepatitis B.

Prevalence of Type 2 diabetes

The overall prevalence of Type 2 diabetes in HCC patients and the cross-sectional controls was 8.4% and 6.3%, respectively ($p = 0.01$) (Table 1). However, as shown in Table 2, in every age group, the prevalence of Type 2 diabetes among HCC patients is not higher than among controls. On the contrary, in the 50- to 59-year-old age group, the prevalence of Type 2 diabetes is higher among CHB controls than among HCC patients. In male patients, the prevalence of Type 2 diabetes was similar between HCC cases and controls. However, in female patients and in patients without cirrhosis, Type 2 diabetes was more frequent among HCC patients than among controls. Unexpectedly, the prevalence of Type 2 diabetes is higher among HCC patients without cirrhosis than among those with cirrhosis (12.1% *vs.* 6.7%, $p < 0.001$).

Type 2 diabetes and HCC risk

As shown in Table 3, in multivariate logistic regression comparing all HCC cases with the hospital cross-sectional controls, no association between Type 2 diabetes and HCC risk was observed. However, in subgroup analysis in female subjects, a significant association between Type 2 diabetes and HCC risk was observed with an AOR of 1.9 (95% CI, 1.1–3.4). In contrast, subgroup analysis in male subjects, no association between Type 2 diabetes and HCC risk was observed. These indicate effect modification (heterogeneity) of gender. The estimated X^2 for homogeneity between AORs in men and women was 5.7. In addition, including an interaction term of gender and Type 2 diabetes in the logistic regression model along with the main effects of gender and Type 2 diabetes revealed a significant interaction between gender and Type 2 diabetes on HCC development ($p = 0.008$). The AOR (95% CI) for the interaction term of diabetes and sex was 0.42 (0.22–0.79), and the β coefficient was −0.88. Besides, including an interaction term of cirrhosis and Type 2 diabetes in the logistic regression model along with the main effects of cirrhosis status and Type 2 diabetes revealed a significant interaction between cirrhosis status and Type 2 diabetes on HCC development ($p = 0.004$). The AOR (95%) for

Table 3. Prevalence of Type 2 diabetes and AOR for the association between Type 2 diabetes and the development of HCC in the presence of CHB: Multivariate logistic regression analyzes

		Men			Women		
Type 2 diabetes	All[1]	All[2]	Without cirrhosis[3]	With cirrhosis[4]	All[5]	Without cirrhosis[6]	With cirrhosis[7]
Cases	93 (8.4%)	73 (7.8%)	28 (9.6%)	45 (7.0%)	20 (11.8%)	14 (25.9%)	6 (5.2%)
Controls	328 (6.3%)	256 (6.7%)	152 (5.7%)	104 (9.0%)	72 (5.3%)	36 (4.0%)	36 (7.9%)
AOR (95% CI)	0.9 (0.7–1.2)	0.8 (0.6–1.1)	1.0 (0.6–1.6)	0.9 (0.6–1.4)	1.9 (1.1–3.4)	5.6 (2.2–14.1)	0.4 (0.2–1.2)

[1]Model 1: Adjusted for age, sex, city of residence, family history of liver cancer, HBeAg status and cirrhosis. [2]Model 2: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and cirrhosis. [3]Model 3: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and platelet. [4]Model 4: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and Child-pugh grade. [5]Model 5: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and cirrhosis. [6]Model 6: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and platelet. [7]Model 7: Adjusted for age, city of residence, family history of liver cancer, HBeAg status and Child-pugh grade.
Abbreviations: AOR, adjusted odds ratio; HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; CI, confidence interval.

the interaction term of diabetes and cirrhosis was 0.45 (0.27–0.77), and the β coefficient was −0.79. Restricted multivariate logistic regression analyses among female patients without cirrhosis indicated a strong association between Type 2 diabetes and HCC risk, with an AOR of 5.6 (95% CI, 2.2–14.1). In female patients with cirrhosis, no association between Type 2 diabetes and HCC risk was observed.

The proportion of patients with a duration of Type 2 diabetes > 5years is significantly higher among HCC cases than among controls ($p < 0.001$) (Table 1). Restricted analyses among diabetic cases and controls showed the AOR of developing HCC were 2.2 (95%CI, 2.2–4.2) for patients diagnosed with a duration of diabetes > 5 years after adjusting age, sex, family history of liver cancer, HBeAg and cirrhosis.

Discussion

In this large case–control study in patients with CHB, Type 2 diabetes was a significant risk factor for HCC in women. The study revealed a high prevalence of Type 2 diabetes among female patients with HCC, but not among male patients with HCC, yielding a two-fold higher risk of HCC for female patients with Type 2 diabetes than for those without Type 2 diabetes. To our knowledge, this finding has not been reported in previous studies.

It is possible that a true relationship exists between Type 2 diabetes and HCC risk in female patients. A gender-based dimorphic pattern for leptin in patients with diabetes mellitus may partially explain the association. There is a strong link between leptin and cancer growth and development,[27] with increasing evidence for the involvement of leptin in HCC development. Higher plasma leptin levels in females with diabetes mellitus may increase HCC risk. Additionally, higher serum ferritin and iron overload in women with diabetes mellitus may also account, in part, for the association between diabetes and HCC risk.[28,29] Liver iron overload is known to be a risk factor for HCC in patients with hemochromatosis, noncirrhotic liver, alcoholic cirrhosis or nonalcoholic steatohepatitis.[30–32] Thus, iron overload might also be a possible link between diabetes mellitus and HCC.

Another important finding of our study is that we observed a significantly higher prevalence of Type 2 diabetes among HCC patients without cirrhosis than among those with cirrhosis (12.1% *vs.* 6.7%, respectively, $p < 0.001$). Accordingly, we noted a marginal significant AOR 1.44 (95% CI, 0.96–2.16) of HCC risk for those with Type 2 diabetes among subjects without cirrhosis. Moreover, restricted multivariate logistic regression analyses stratified by sex and cirrhosis status indicated that for female CHB patients without cirrhosis, those who had Type 2 diabetes had an approximately six times higher risk of HCC than did those without Type 2 diabetes ($p < 0.001$). Our finding indicates a synergistic association between Type 2 diabetes and cirrhosis status on HCC risk. Because of the small number of female HCC patients without cirrhosis in our study, this result should be considered with caution. However, this result is in consistent with a Taiwan cohort study, which reported the synergistic association between Type 2 diabetes and HCC risk among patients with both diabetes and hepatitis B infections, compared to those with the viral infections but without diabetes.[33] Interestingly, several recent studies showed that HCC in metabolic syndrome and nonalcoholic fatty liver disease often develop in the absence of apparent cirrhosis.[34–36] These results also support our finding that Type 2 diabetes is an additional HCC risk in the absence of cirrhosis in patients with CHB. High insulin concentration and insulin resistance preceding cirrhosis and HCC in Type 2 diabetes might play a key role in the HCC carcinogenesis. Thus, the discovery of a high prevalence of Type 2 diabetes in noncirrhotic HBV-related HCC patients should not be surprising.

We also noted a significant association between diabetes duration and HCC risk in our study population, a two-fold higher HCC risk for subjects with diabetes duration > 5 years than for those with a shorter duration. This finding is in agreement with newly published study by Hassan *et al.*,[37] which reported that the magnitude of association between diabetes and HCC increased as the duration of diabetes increased. The result suggested that the association between diabetes and HBV-related HCC risk was not a temporal relationship.

Our study has several advantages over previous studies. First, to the best of our knowledge, this is the largest case–control study published to date that investigates the impact of Type 2 diabetes on the development of HCC in the presence of CHB. Second, cross-sectional inpatient CHB controls in our study largely represents the population from which the HBV-related HCC cases arose. In areas of high HBV endemicity, persons with cirrhosis have an approximately three-fold higher risk for HCC than those with chronic hepatitis but without cirrhosis and a 16-fold higher HCC risk than the inactive carrier.[17] So, most HBV-related HCC arose from CHB with or without cirrhosis but not from inactive HBsAg carrier. Third, some possible confounding factors, such as alcohol consumption, family history of HCC, city of residence, HBeAg and cirrhosis status were evaluated in our study.

There are certain limitations to the study. First, overweight and obesity were not included in our study because of the overweight and duration was difficult to ascertain, subject to recall bias and be confounded by ascites in subjects with cirrhosis. Second, not all HCC and cirrhosis subjects were diagnosed histologically. However, we used a standard clinical criteria combined with well-defined hepatic imagine method for the diagnosis of HCC and cirrhosis patients without a liver biopsy.[23] Moreover, all of the patients in our study were followed and revaluated every 1–3 months in the outpatient division of our hospital.

In conclusion, we report the first independent association between Type 2 diabetes and an increased HCC risk in female patients with CHB. This indicates besides male CHB

patients and patients with cirrhosis, female CHB patients with Type 2 diabetes are of a high HCC risk population and should be considered for HCC close surveillance program. Further clinical and basic studies are necessary to confirm the gender difference in HCC risk and to elucidate the underlying mechanisms of this risk.

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CLINICAL STUDIES

Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors

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Keywords
ablation – hepatocellular carcinoma – percutaneous ethanol injection – prognostic factor – recurrence – survival – treatment outcome

Abbreviations
AFP-L3, lectin-reactive AFP; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence intervals; CT, computed tomography; DCP, des-γ-carboxy-prothrombin; HBs-Ag, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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Abstract

Background: Ethanol injection is the best-known image-guided percutaneous ablation for hepatocellular carcinoma (HCC) and a well-tolerated, inexpensive procedure with few adverse effects. However, there have been few reports on its long-term results. *Aims:* We report a 20-year consecutive case series at a tertiary referral centre. *Methods:* We performed 2147 ethanol injection treatments on 685 primary HCC patients and analysed a collected database. *Results:* Final computed tomography demonstrated complete ablation of treated tumours in 2108 (98.2%) of the 2147 treatments. With a median follow-up of 51.6 months, 5-, 10- and 20-year survival rates were 49.0% [95% confidence interval (CI) = 45.3–53.0%], 17.9% (95% CI = 15.0–21.2%) and 7.2% (95% CI = 4..5–11.5%) respectively. Multivariate analysis demonstrated that age, Child–Pugh class, tumour size, tumour number and serum alpha-fetoprotein level were significant prognostic factors for survival. Five-, 10- and 20-year local tumour progression rates were 18.2% (95% CI = 15.0–21.4%), 18.4% (95% CI = 15.2–21.6%) and 18.4% (95% CI = 15.2–21.6%) respectively. Five-, 10- and 20-year distant recurrence rates were 53.5% (95% CI = 49.4–57.7%), 60.4 (95% CI = 56.3–64.5%) and 60.8% (95% CI = 56.7–64.9%) respectively. There were 45 complications (2.1%) and two deaths (0.09%). *Conclusions:* Ethanol injection was potentially curative for HCC, resulting in survival for more than 20 years. This study suggests that new ablation therapies will achieve similar or even better long-term results in HCC.

Hepatocellular carcinoma (HCC) is the fifth most common malignant neoplasm in the world. Only 20% of HCC patients are candidates for resection (1). Furthermore, recurrence is frequent even after curative resection. Liver transplantation is restricted by donor shortage. Thus, various non-surgical therapies have been introduced (2). Among these, image-guided percutaneous ablation is considered best for early-stage HCC.

The most studied percutaneous ablation is ethanol injection. Ethanol injection is a well-tolerated, inexpensive procedure with few adverse effects and has been considered the standard against which any new ablation therapy should be compared (2). Although ethanol injection was introduced into clinical practice in the 1980s (3, 4), few reports of its long-term results have been published (5–8). We report here a 20-year consecutive case series at a tertiary referral centre. This study documents the largest number of ethanol injection treatments at a single institution. Findings in this 20-year experience may be extrapolated to other ablation therapies, such as radiofrequency ablation, in which such long-term outcomes are not yet available (9).

Patients and methods

Indications for ethanol injection

Ethanol injection was performed in patients satisfying the following criteria: (i) ineligible for resection or transplantation, or had refused surgery; (ii) no extrahepatic metastasis or vascular invasion. Exclusion criteria were as follows: (i) tumour was not visualized

by ultrasonography or not accessible percutaneously; (ii) total bilirubin level ≥ 3.0 mg/dl; (iii) platelet count $<40 \times 10^9$/L; (iv) prothrombin activity <35%; (v) refractory ascites. In general, we performed ethanol injection on patients with Child–Pugh class A or B, with 3 or fewer tumours ≤ 3 cm in diameter. We performed ethanol injection on patients beyond these conditions, however, who were likely to benefit from the procedure for possible cure or prolongation of life. No patients were excluded solely because of tumour location (10). Informed consent was obtained from each patient. This study was conducted according with the Helsinki Declaration of 1975 and approved by the Institutional Review Board.

Patients

In this cohort study, we analysed a prospectively collected computerized database. Between 1985 and 2005, 2735 HCC patients were admitted to the Department of Gastroenterology, University of Tokyo (Fig. 1). At initial hospitalization, 1615 had primary HCC and the remaining 1120 had recurrent HCC. The recurrent HCC patients had undergone therapies other than ethanol injection for primary HCC.

Of the 1615 patients with primary HCC, 1459 (90.3%) underwent percutaneous ablation as the initial treatment, including ethanol injection. The remaining 156 patients received other therapies: transarterial chemoembolization for 123 patients with multinodular or large tumours that could not be treated by ablation therapies; hepatic resection for 18 with good liver function who consented to an operation; chemotherapy for four with vascular invasion or extrahepatic metastasis; and best supportive care for 11 with decompensated cirrhosis or poor general condition.

Of the 1459 patients treated by percutaneous ablation, 685 underwent ethanol injection, 122 underwent microwave ablation, and the remaining 652 radiofrequency ablation. The type of percutaneous ablation performed varied with the date of treatment. We started ethanol injection in December 1985, microwave ablation in October 1995 and radiofrequency ablation in February 1999 (11). Between October 1995 and February 1999, both ethanol injection and microwave ablation were performed. Microwave ablation was chosen for patients who had better liver function and whose tumour was located in a position where the electrode could be inserted and held safely. Since February 1999, both ethanol injection and radiofrequency ablation have been performed. Between April 1999 and January 2001, 232 patients with three or fewer tumours, each ≤ 3 cm in diameter, and Child–Pugh class A or B were entered into a randomized controlled trial (12). Patients outside these inclusion criteria were mostly treated by radiofrequency ablation. After this trial, radiofrequency

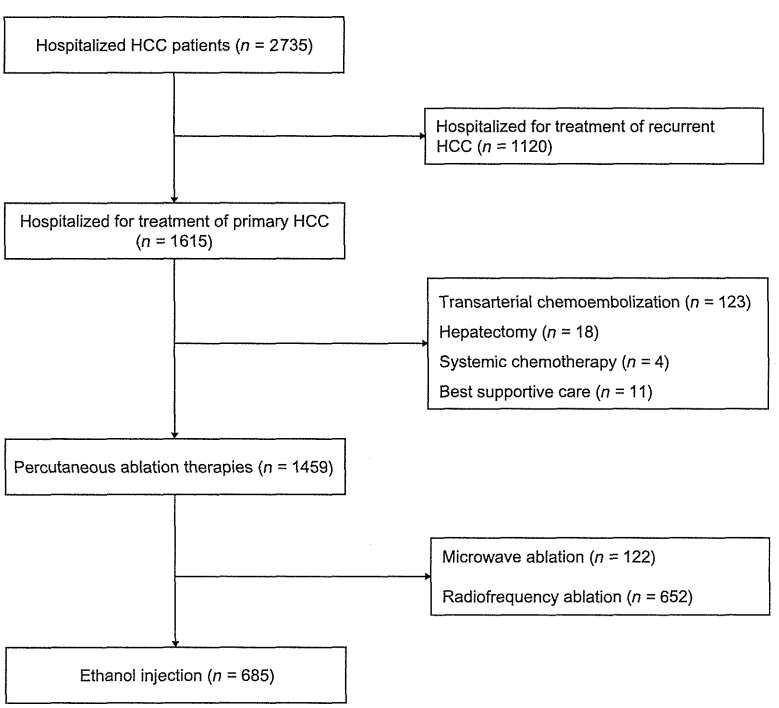

Fig. 1. Flow of patients in this study. HCC, hepatocellular carcinoma

ablation was generally the treatment of choice, and ethanol injection was used only in those unsuitable for radiofrequency ablation: those with either enterobiliary reflux or tumour adhesion to the gastrointestinal tract.

Hepatocellular carcinoma was diagnosed based on typical imaging findings of early phase enhancement and late phase contrast washout on computed tomography (CT) (13). HCC diagnosis was also confirmed by biopsy in 630 (92.0%) of the 685 patients with primary HCC treated by ethanol injection. A total of 587 (85.7%) were diagnosed as having cirrhosis.

In general, chemoembolization was combined with ethanol injection in patients with either ≥ 4 tumours or those with two or three tumours at least one of which is >3.0 cm in diameter. The combination of chemoembolization with ethanol injection was performed in 186 patients.

Treatment methods

Preoperative planning including ultrasound examination and evaluation of all imaging findings was performed to identify the tumours and to determine the access route. The procedure was performed according to an institutional protocol and under the supervision of experienced physicians who had performed this treatment more than 200 times. The precise techniques of ethanol injection are described elsewhere (12). Briefly, all procedures were performed percutaneously under ultrasound guidance. Artificial pleural effusion or artificial ascites method is much less frequently used in ethanol injection compared with radiofrequency ablation, because the procedure is necessary to be repeated several times. Since 1990, we have used two or three needles to inject ethanol into several sites in one procedure (12). Ethanol injection was performed twice per week. The procedure was repeated until ethanol appeared to have been injected throughout the tumour. To judge a timing to stop repetition of injecting ethanol and to order a CT scan, we considered total volume of injected ethanol and change of echogenicity. The general guideline for the necessary volume of injected ethanol was calculated according to the following numerical expression, $V = (4/3)\ \pi\ (r + 0.5)^3$, where V (in millilitres) is the volume of ethanol and r (in centimetres) is the radius of the tumour; 0.5 is added to provide a safety margin, which is based on the concept that some surrounding liver parenchyma all around the tumour as well as the tumour itself must be ablated (5).

A CT scan was then performed 1–3 days after the procedure to evaluate technique effectiveness (14). Complete ablation was defined as hypoattenuation of the entire tumour. When the presence of unablated tumour portions was suspected, a few more procedures were performed. We did not predefine the number of procedures in a treatment. The ethanol injection treatment was generally continued until CT demonstrated the entire tumour necrosis.

Follow-up

Follow-up investigations consisted of CT, ultrasonography and measurement of serum α-fetoprotein (AFP), des-γ-carboxy-prothrombin (DCP) (since April 1993) levels and lectin-reactive AFP (AFP-L3) (since July 1997) every 4 months. Local tumour progression was defined as appearance of viable tumour touching the original tumour (14) and distant recurrence as emergence of tumour(s) separate from the primary site. Ethanol injection was used for recurrence if the patient still met the indication criteria. If multiple recurrences were not treatable with ethanol injection, chemoembolization was generally performed.

Statistical analyses

This study is a report of a consecutive case series. All ethanol injection treatments performed on primary HCC patients at the Department of Gastroenterology, University of Tokyo between 1985 and 2005 were included. Data are presented as mean ± SD for quantitative variables, and as absolute frequencies for qualitative variables.

A 'procedure' was defined as a single intervention episode that consisted of one or more ablations performed on tumours, and a 'treatment' as the completed effort to ablate tumours. A treatment consisted of several procedures (14). 'Technique effectiveness' rate was defined as the percentage of successfully eradicated macroscopic tumours as evidenced at CT scan after the last procedure (14). In cases in which there was Lipiodol deposit inside the tumour because of the combination of chemoembolization with ethanol injection, we judged that the tumour had been successfully eradicated if it was surrounded with completely non-enhanced tissue in final CT.

Overall survival was calculated in the 685 primary HCC patients. Survival curves were generated using the Kaplan–Meier method. In addition to overall survival, subgroup analyses were performed with clinical characteristics including tumour size, tumour number and Child–Pugh class. Recurrence was evaluated in 591 patients in whom ethanol injection was performed with curative intent. All tumours were treated by ethanol injection in those patients. The remaining 94 patients were excluded from the recurrence analysis because some small tumours had been left untreated by ethanol injection on account of detection failure by ultrasonography. Recurrence rates were calculated using the Gaynor method (15). All time estimates were made from the date of the first ethanol injection. The follow-up was finalized at either death or the last visit to the outpatient clinic before December 31 2010. Transplanted patients were censored from this study at the date of transplantation.

The prognostic relevance of baseline variables (Table 1), the combination of chemoembolization,

Table 1. Baseline characteristics of the 685 Patients undergoing percutaneous ethanol injection for primary hepatocellular carcinoma

Variable	
Age (years)	64.0 ± 8.9
Males, *n* (%)	502 (73.3)
Viral infection*	
HBs-Ag positive, *n/N* (%)	64/685 (9.3)
Anti-HCV positive, *n/N* (%)	570/673 (84.7)
Both positive, *n/N* (%)	11/673 (1.6)
Both negative, *n/N* (%)	52/673 (7.7)
Alcohol consumption >80 g/day, *n* (%)	143 (20.9)
Ascites, *n* (%)	122 (17.9)
Encephalopathy, *n* (%)	44 (6.5)
Albumin (g/dl)	3.55 ± 0.50
Total bilirubin (mg/dl)	0.96 ± 0.536
Prothrombin time (%)	71.6 ± 15.9
Platelet count ($\times 10^4/mm^3$)	10.3 ± 4.6
AST (IU/L)	80.6 ± 48.2
ALT (IU/L)	79.2 ± 61.9
Child–Pugh class, *n* (%)	
A	425 (62.1)
B	228 (33.3)
C	32 (4.6)
Tumour size (cm)	2.83 ± 1.47
Tumour number	2.0 ± 1.7
Serum AFP (ng/ml), *n* (%)	
≤100	525 (76.6)
101–400	95 (13.9)
>400	65 (9.5)
Serum DCP (mA U/ml), *n* (%)†	
≤100	428 (82.8)
101–400	49 (9.5)
>400	40 (7.7)
Serum AFP-L3 (%), *n* (%)‡	
≤15	193 (86.2)
15.1–40	16 (7.1)
>40	15 (6.7)

*Anti-HCV was not tested in 12 patients.

†Serum DCP level was not measured in 168 patients.

‡Serum AFP-L3 level was not measured in 461 patients.

HBs-Ag, hepatitis B surface antigen; HCV, hepatitis C virus; AFP, α-fetoprotein; DCP, des-gamma-carboxy-prothrombin; AFP-L3, lectin-reactive α-fetoprotein.

Data are expressed as mean ± standard deviation.

HCC recurrence and the number of ethanol injection sessions to survival was analysed by univariate and multivariate models. The prognostic relevance of baseline variables (Table 1), the combination of chemoembolization and the number of ethanol injection sessions to local tumour progression and distant recurrence was also analysed by univariate and multivariate models. In multivariate analysis, we evaluated models including Child–Pugh class and excluding its components to avoid multicollinearity. Serum DCP and AFP-L3 levels were excluded from the multivariate model because of absence of data from 168 and 461 patients respectively. Some continuous variables in which log-linearity could not be assumed were transformed into categorical variables. Variables with a P value <0.05 determined by univariate comparison were subjected to multivariate analysis. A stepwise variable selection was performed with Akaike Information Criteria in multivariate analysis. Results were expressed as hazard ratios with corresponding 95% confidence intervals (CI), with P values from the Wald test. All significance tests were two-tailed, and differences with a P value <0.05 were considered statistically significant.

Complications were defined according to the guidelines of the Society of Interventional Radiology (16).

Results

Antitumour effect

We performed 2147 ethanol injection treatments, comprising 13 526 procedures. Thus, procedure number per treatment was 6.3 ± 2.6. The total volume of injected ethanol per treatment was 40.9 ± 16.3 ml. Many patients received iterative ethanol injection treatments for recurrence. A total of 108 patients underwent ethanol injection treatment once, 118 patients twice, 196 patients 3 times, 153 patients 4 times, 71 patients 5 times, 28 patients 6 times, 8 patients 7 times and 3 patients 8 times.

Technique effectiveness rate was 98.2% (2108/2147 treatments). It was similar between the initial ethanol injection treatments and the other ethanol injection treatments for recurrence ($P = 0.397$). Complete ablation of the tumour was achieved in 675 (98.5%) of the 685 initial treatments and in 1433 (98.0%) of the 1462 other treatments. However, technique effectiveness rate significantly differed with tumour size ($P = 0.002$). No apparent viable portions remained in 758 (99.0%) of 766 treatments for tumours ≤2.0 cm in diameter, in 704 (98.4%) of 717 treatments for tumours 2.1–3.0 cm, in 570 (97.9%) of 582 treatments for tumours 3.1–5.0 cm and in 76 (92.7%) of 82 treatments for tumours >5.0 cm.

Survival

Table 1 shows clinical characteristics of the 685 patients. A total of 136 patients (19.9%) were older than 75 years. In all, 180 patients had tumours ≤2.0 cm in diameter, 274 had tumours 2.1–3.0 cm, 192 had tumours 3.1–5.0 cm and 39 had tumours >5.0 cm. A total of 367 patients had one tumour, 238 patients had 2 or 3 tumours and 80 had 4 or more tumours.

As of December 2010 (with a median follow-up of 51.6 months), 70 patients (10.2%) remained alive, 52 (7.6%) were lost to follow-up and 563 (82.2%) had died. Of the 685 patients, two were transplanted. The number of patients who survived longer than 5, 10 and 20 years after the first ethanol injection treatment was 305, 97 and 3 respectively. The cause of death was HCC

in 297 patients (52.8%), liver failure in 129 (22.9%), upper gastrointestinal bleeding in 30 (5.3%), complications related to the procedure in 2 (0.4%), liver-unrelated diseases in 84 (14.9%) and undetermined in 21 (3.7%).

The 1-, 3-, 5-, 10-, 15- and 20-year survival rates of all 685 patients were 91.0% (95% CI = 88.9–93.2%), 67.6% (95% CI = 64.1–71.3%), 49.0% (95% CI = 45.3–53.0%), 17.9% (95% CI = 15.0–21.2%), 8.6% (95% CI = 6.4–11.7%) and 7.2% (95% CI = 4.5–11.5%) respectively (Fig. 2; Table 2). Survival rates significantly differed with tumour number ($P = 0.0001$), tumour size ($P = 0.0001$) and Child–Pugh class ($P = 0.0001$). In patients with 1–3 tumours, all ≤ 3 cm, and in Child–Pugh class A or B, the 5-year survival rate was 59.5% (95% CI: 54.7–64.7%).

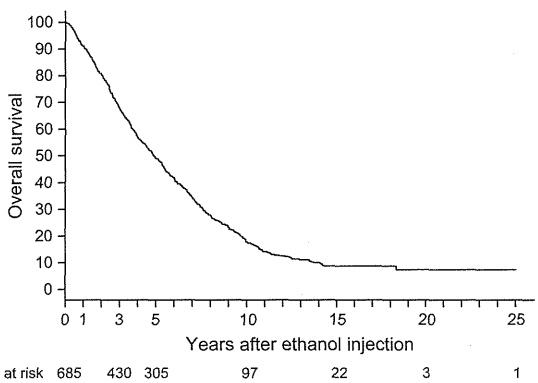

Fig. 2. Overall survival in 685 primary hepatocellular carcinoma patients who underwent ethanol injection.

Univariate analysis indicated that 13 of the 22 variables were relevant to survival. In multivariate analysis, a model that contained age, antibody to hepatitis C virus (anti-HCV), Child–Pugh class, tumour size, tumour number and serum AFP level was selected (Table 3).

Survival rates significantly differed with the time period in which the first ethanol injection was performed ($P < 0.0001$; Fig. 3). In 109 patients who underwent ethanol injection between 1985 and 1991, the 5-year survival rate was 30.3% (95% CI = 22.7–40.5%), whereas it was 51.2% (95% CI = 46.8–55.9%) in 476 patients between 1992 and 1998, and 61.1% (95% CI = 51.3–72.8%) in 100 patients between 1999 and 2005.

Recurrence

Recurrence developed in 449 patients. Local tumour progression alone was found in 61 patients, local tumour progression with distant recurrence in 44 and distant recurrence alone in 344. Of these 344 patients, eight had recurrence in extrahepatic sites: five had lymph node metastasis, one had lung metastasis, one had bone metastasis and the remainder had both lymph node and lung metastasis. Of the 449 patients, the first recurrence was treated by iterative ethanol injection in

Table 2. Survival of patients undergoing ethanol injection, based on tumour number, tumour size and Child–Pugh class

Grading	*n*	Survival (%) 3-Year	5-Year	10-Year	15-Year	20-Yar	Median (years)	*P* value
Overall survival	685	67.6	49.0	17.9	8.6	7.2	4.9	–
Tumour number								
Solitary	367	72.0	56.5	24.6	12.1	9.7	5.8	0.0001
2–3	232	71.5	46.3	12.9	5.9	–	4.7	
≥4	86	37.6	23.8	2.5	1.3	–	2.6	
Tumour size								
≤2.0 cm	240	83.6	63.8	27.6	12.3	6.1	6.9	0.0001
2.1–3.0 cm	221	68.0	47.9	15.0	10.7	10.7	4.8	
>3.0 cm	224	50.2	34.4	10.1	3.5	3.5	3.1	
Child–Pugh class								
A	425	77.3	58.7	24.4	12.5	10.4	6.2	0.0001
B	228	53.9	35.5	8.1	3.0	–	3.5	
C	32	37.5	18.8	3.1	–	–	1.9	
Combination of tumour number, tumour size, and Child–Pugh class								
Solitary, ≤3 cm	275	77.5	62.2	28.8	14.5	10.8	6.8	–
Solitary, ≤3 cm, Child–Pugh A	185	84.9	69.2	36.7	20.2	15.1	7.6	–
1–3 tumours, ≤3 cm	419	78.6	58.0	23.5	12.2	9.1	6.1	–
1–3 tumours, ≤3 cm, Child–Pugh A/B	402	80.5	59.5	24.3	12.8	9.6	6.2	–
Satisfied the indication criteria of surgical resection proposed in the BCLC protocol*	121	86.3	72.8	31.1	14.8	–	7.2	–

*Child–Pugh class A with a normal level of bilirubin, no significant portal hypertension and a single HCC.
BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

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Table 3. Multivariate analysis of variables relevant to survival, local tumour progression and distant recurrence

Variable	Multivariate analysis Hazard ratio (95% CI)	*P* value
Survival		
Age (per year)	1.03 (1.02–1.04)	<0.0001
Anti-HCV-positive	0.81 (0.69–0.94)	0.006
Child–Pugh class		
A	1	
B	2.01 (1.66–2.44)	<0.0001
C	3.11 (2.08–4.65)	<0.0001
Tumour size (cm)		
≤2.0	1	
2.1–3.0	1.26 (1.00–1.58)	0.051
3.1–5.0	1.51 (1.18–1.93)	0.001
>5.0	2.31 (1.61–3.31)	<0.0001
Tumour number		
solitary	1	
2–3	1.10 (0.90–1.35)	0.34
≥4	2.11 (1.59–2.78)	<0.0001
Serum AFP (ng/dl)		
≤100	1	
101–400	1.47 (1.14–1.90)	0.003
>400	2.16 (1.57–2.97)	<0.0001
Local tumour progression		
Tumour size (cm)		
≤2.0	1	
2.1–3.0	1.47 (1.15–1.88)	0.002
3.1–5.0 vs. ≤2.0	1.30 (0.97–1.75)	0.08
>5.0 vs. ≤2.0	2.81 (1.64-4.82)	0.0002
Distant recurrence		
Tumour size (cm)		
≤2.0	1	
2.1–3.0	1.42 (1.11–1.82)	0.006
3.1–5.0	1.28 (0.95–1.72)	0.10
>5.0	2.48 (1.43–4.28)	0.001
Tumour number		
solitary	1	
2–3	1.47 (1.16–1.85)	0.001
≥4	2.12 (1.36–3.28)	0.0008

AFP, α-fetoprotein; CI, confidence interval; HCV, hepatitis C virus.

399 (88.8%), chemoembolization in 44 (9.8%), systemic chemotherapy in three (0.7%) and best supportive care in three (0.7%).

The 1-, 3-, 5-, 10-, 15- and 20-year rates of local tumour progression with or without distant recurrence were 7.9% (95% CI = 5.7–10.0%), 15.6% (95% CI = 12.6–18.6%), 18.2% (95% CI = 15.0–21.4%), 18.4% (95% CI = 15.2–21.6%), 18.4% (95% CI = 15.2–21.6%) and 18.4% (95% CI = 15.2–21.6%) respectively (Fig. 4). Univariate analysis demonstrated that three variables were relevant to local tumour progression, whereas multivariate analysis indicated that only tumour size was significantly related to local tumour progression (Table 3).

The 1-, 3-, 5-, 10-, 15- and 20-year rates of distant recurrence without local tumour progression were 17.1% (95% CI = 14.0–20.1%), 42.6% (95% CI = 38.6–46.7%), 53.5% (95% CI = 49.4–57.7%), 60.4% (95% CI = 56.3–64.5%), 60.8% (95% CI = 56.7–64.9%) and 60.8% (95% CI = 56.7–64.9%) respectively. Univariate analysis demonstrated that five variables were relevant to distant recurrence, whereas multivariate analysis indicated that tumour size and tumour number were significantly related to distant recurrence without local recurrence (Table 3).

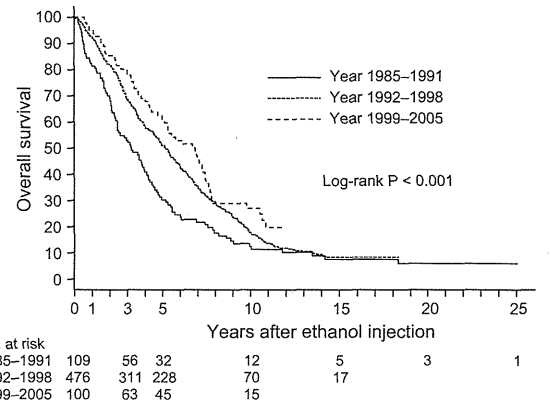

Fig. 3. Survival according to the time period in which the first ethanol injection was performed (1985–1991 vs. 1992–1998 vs. 1999–2005)

Complications

Table 4 shows complications encountered. The incidence rates per treatment and per procedure were 2.1% (45 of 2147) and 0.33% (45 of 13 526) respectively. A patient died of multiple organ dysfunction syndrome caused by procedure-related hemoperitoneum. The tumour was not on the surface but inside the liver. The patient did not have marked bleeding tendency. The other developed myocardial infarction, resulting in death during the procedure. The treatment mortality rate was 0.06%.

Discussion

This study describes a 20-year experience with ethanol injection at a high-volume centre. We performed 2147 ethanol injection treatments on the 685 primary HCC patients, showing that ethanol injection has a high antitumour effect. Tumours were judged to have been completely ablated by final CT imaging in 98.2% of the treatments. The complete response rate may be higher in this study than others (17, 18), probably because we did not predefine the number of procedures in a treatment. We generally repeated the procedure until CT demonstrated complete tumour necrosis. Many other studies limited the procedure number of ethanol injection. Complete tumour ablation has been reported to relate to improved survival (19).

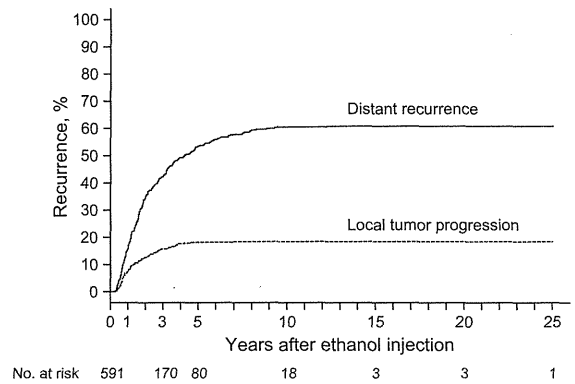

Fig. 4. Local tumour progression or distant recurrence in patients who underwent ethanol injection.

Table 4. Complications in 2147 treatments of ethanol injection for hepatocellular carcinoma

Complication	Number
Neoplastic seeding	9
Hemoperitoneum	9
Hemobilia	6
Liver abscess	6
Symptomatic pleural effusion	3
Massive hepatic infarction	3
Biliary cast	2
Hemothorax	2
Abnormal decrease in blood coagulation factor VIII	2
Biloma	1
Biliary bronchial fistula	1
Myocardial infarction	1

This study showed that ethanol injection could achieve long-term survival over 20 years. Ninety-seven patients survived for more than 10 years and three for more than 20 years. Both tumour factors and liver function were relevant to survival. In addition, age was among the prognostic factors. In this study, 19.9% were older than75 years, which may have resulted in the higher percentage (14.9%) of liver-unrelated deaths compared with other studies. Ant-HCV positivity was a good prognostic factor in this study.

Survival in ethanol injection appears to have improved with times. This is probably because of advances in imaging techniques, such as ultrasound and CT, more refined skills and greater experience in ablation and innovations in the treatment of underlying liver diseases.

Hepatocellular carcinoma frequently recurred after ethanol injection. Most recurrences were, however, not local tumour progression but distant recurrence. Frequent recurrence is not specific to ethanol injection. After hepatic resection, the tumour recurrence rate exceeds 70% at 5 years (20, 21). In this study, periodic follow-up detected most recurrence at limited stage. Ethanol injection was performed again for first recurrence in 88.8% of the cases. In hepatic resection, the rate of repeat resection for first recurrence has been reported to range from 10.4 to 30.6% (21, 22). As ethanol injection is less invasive than hepatic resection, iterative ethanol injection can be performed for recurrence more easily.

Ethanol injection was a safe procedure, although many patients in this study were at risk for surgical treatment because of advanced cirrhosis or other comorbidities. Only 121 (17.7%) of the 685 patients satisfied the indication criteria of surgical resection proposed in the BCLC (Barcelona Clinic Liver Cancer) protocol (23) and were, thus, considered good candidates for surgical resection. Other investigators also reported low complication rates of 0–3.2% (6–8, 24). For hepatic resection, morbidity rates have been reported to be 38–47% even in recent studies (25–27).

Radiofrequency ablation has steadily replaced ethanol injection (11). At our institution, radiofrequency ablation is currently the first option for percutaneous ablation (28). Several randomized controlled trials including ours (12, 18, 29, 30) demonstrated more reliable local antitumour effect and higher survival. Our 10-year outcome of radiofrequency ablation (28) appears superior to this 20-year outcome of ethanol injection. In addition, radiofrequency ablation requires fewer treatment sessions and shorter hospitalization.

A meta-analysis showed, however, that ethanol injection did not differ from radiofrequency ablation for tumours ≤ 2 cm in diameter (31). A recent randomized controlled trial also demonstrated similar 5-year survival between the two ablations (32). Ethanol injection is at least more feasible and cheaper than radiofrequency ablation.

Surgical resection has been considered the treatment of first choice for HCC. Our first option for resectable tumours was also surgery. However, most patients who came to our department declined surgical resection. Thus, some patients in this study underwent ethanol injection not because of unresectable tumour but because of refusal of surgery. Those who preferred surgery would have gone directly to the surgical department, which has extensive experience in hepatic resection (27).

It is not easy to compare outcomes between ethanol injection and surgical resection. Indications are different between the two treatments. Furthermore, indications for each treatment are different from institution to institution. Thus, a case adjudged to be treatable by ethanol injection or surgical resection at an institution may not be given the same treatment at another. The best-known indication criteria may be those proposed in the BCLC protocol (23), which states that surgical resection should be restricted to patients with performance status 0, Child–Pugh class A, single HCC, normal portal pressure and normal serum bilirubin level. In patients satisfying

those criteria, the 5-year survival rate is expected to be >70% (20). In this study, 5-year survival rate of the patients satisfied the criteria was 72.8%, which appears satisfactory when compared with outcomes following surgical resection. Furthermore, in patients with solitary HCC, ≤ 3 cm in diameter, and Child–Pugh A, 5- and 10-year survival rates were 69.2% and 36.7% respectively. In patients treated by surgical resection, 5- and 10-year survival rates were 34.4–70.0% and 10.5–52.0% respectively (22, 33–39). Although this is an observational study with no control, survivals following ethanol injection appear comparable to those reported following surgical resection.

A randomized controlled trial showed no significant difference in survival between ethanol injection and surgical resection (40). Several non-randomized controlled trials also reported similar overall survival between the two treatments (5–7, 40–43), whereas others reported higher survival with resection (44). Further studies are necessary to resolve this issue of comparing ablation with resection.

We made strenuous efforts to standardize the procedure of ethanol injection because many physicians performed ethanol injection at our institution. In addition to proficient practice of ethanol injection, detailed preoperative planning, cautious postoperative evaluation of therapeutic effect and careful follow-up are vital to achieve satisfactory outcomes.

Source population in this study may represent selection bias, as we performed ethanol injection on most patients who were hospitalized at our department; however, many patients with unfavourable tumour conditions for ethanol injection might not have been referred to us. Therefore, caution is required when extrapolating our findings to the general population of HCC patients.

A second limitation is that study population cannot be clearly defined. This study was based on daily clinical practice over a 20-year period. Indication criteria of ethanol injection changed over time, mainly because of the introduction of the other ablations: microwave ablation and radiofrequency ablation. Furthermore, various treatments besides percutaneous ablations were available for HCC, such as surgical resection and chemoembolization, with frequently overlapping indications.

One further limitation is the fact that this was a single-centre study. To extrapolate the findings in this study to patients at other institutions, consideration should be given to differences in the indications, methods, expertise, performance of available ultrasound and CT equipment and others. Treatment outcome may be influenced by the physicians' expertise and the institution's volume of care. We performed over 2000 ethanol injection treatments, which may represent a much greater number of treatments than those in most other institutions.

In conclusion, our 20-year experience shows that ethanol injection was potentially curative, resulting in long-term survival over 20 years. Findings in this study may suggest that other ablation therapies, such as radiofrequency ablation, will achieve similar or even better long-term results in HCC.

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