

A case-control study of hepatitis B and C virus infection as risk factors for hepatocellular carcinoma in Henan, China

Jian-Ying Zhang,^{a,b} Min Dai,^a Xiao Wang,^a Wei-Quan Lu,^a Dong-Sheng Li,^a Mei-Xi Zhang,^a Ke-Juan Wang,^a Li-Ping Dai,^a Sheng-Gao Han,^a Yuan-Fang Zhou^a and Hui Zhuang^c

Background Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and is particularly prevalent in China. China is also a hyperendemic area for hepatitis B virus (HBV) infection. Although a strong association between HBV infection and HCC has been established previously, the role of hepatitis C virus (HCV) infection and the interaction between HBV and HCV in the development of HCC has not been adequately explored. The major objective of this study is to determine the relationship between HBV or HCV infection and HCC by use of case-control study in Henan, China.

Method In all, 152 HCC patients and 115 control patients were collected from four hospitals in Henan, China between January 1994 and October 1995. The demographic characteristics of the two groups were comparable. In further analysis, a 1:1 pair-matched case-control study was performed. Of 152 HCC patients, 113 were randomly selected to be pair-matched by sex and age (± 5 years) to controls with non-hepatic disease. All the cases and controls were interviewed during hospitalization by two specially trained interviewers using a standard questionnaire. All sera were tested for HBV and HCV markers. Odds ratios (OR) and 95% CI for HCC risk factors were calculated by logistic regression model controlling for possible confounding factors such as sex and age. The multivariate analysis was done on the basis of the univariate analysis.

Results The results of this study indicated that the prevalence of hepatitis B surface antigen (HBsAg) and antibody to HCV (anti-HCV) were much higher in HCC patients (63.2% and 11.2% respectively) than in the control patients (5.2%, 3.5%). The difference between two groups was significant ($P < 0.05$). Risk factor analysis revealed that both HBV and HCV infection were important factors for HCC in Henan, China and HBV appeared to have a key role in the development of HCC. Odds ratios of HBsAg and HBV infection were 28.82 (95% CI: 11.18-78.78) and 31.22 (95% CI: 13.86-72.15), respectively. Moreover, the risk of developing HCC increased significantly and showed an additive effect when both viral markers of HBV and HCV infection were considered (OR = 42.85). Results from the 1:1 pair-matched case-control study also showed that HBV infection was an important risk factor for HCC, which was consistent with the results from the group-matched case-control study.

Conclusion This is the first reported case-control study of HCC in Henan, China. This study provides further evidence that chronic HBV infection is strongly associated with the development of HCC among this population. Our results have demonstrated that HCV and HBV infection are independent and probably additive risk factors for HCC.

Keywords Hepatocellular carcinoma, prevalence, risk factor, hepatitis B virus, hepatitis C virus, case-control study

Accepted 11 November 1997

^a Department of Epidemiology, Henan Medical University, Zhengzhou, Henan 450052, PR of China

^b Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA.

^c Department of Epidemiology, Beijing Medical University, Beijing 100083, PR of China.

Reprint requests to: Dr Jian-Ying Zhang, SBR6, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, and is particularly prevalent in Africa and Asia.^{1,2} In China, HCC is the third leading cause of cancer death, after cancer of the stomach and oesophagus. Geographical variation in the mortality rate of this cancer is also evident in China. The counties and provinces or autonomous regions with higher mortality rates are arranged in a band running from north to south along the southeastern seacoast.³ Henan province, which is located in central China, has a population of about 90 million people. In 1992 the Henan Tumor Research Institute reported a mortality rate for malignant liver tumour in Henan province of 22.78 per 100 000 people annually. This rate is relatively high compared to the standardized mortality rate for HCC in China. In China and other countries the principal aetiological factors for HCC have been identified as hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, dietary exposure to aflatoxin, alcohol drinking and oral contraceptive use. On a worldwide basis, chronic infection with HBV has been considered to be the most important risk factor for HCC, being implicated in about 80% of cases. Since diagnostic systems for HCV infection have become available, HCV infection has become recognized as a more important aetiological factor in the pathogenesis of HCC than HBV in some parts of the world and HBV infection is implicated in less than 30% of HCC cases in low-endemicity countries. In those countries the incidence of HCC has increased during the past decade, although cases of HBV infection have remain unchanged or are instead increasing. It has recently been shown that the HCC cases which are negative for HBV infection are often linked to HCV infection.^{4,5} Up to now we do not know which factor is the most important risk factor in the development of HCC in Henan. Henan is a hyperendemic area for HBV infection. Although a strong association between HBV infection and HCC has been established by many studies in China and other countries, the role of HCV infection, and the interaction between HBV and HCV in the development of HCC in Henan has not been adequately explored. The major objective of this study is to determine the relationship between HBV or HCV infection and HCC in Henan using a case-control study.

Materials and Methods

In this study, 152 patients with HCC were recruited among patients admitted between January 1994 and October 1995 to four hospitals in Henan province, including the First and Second Teaching Hospital of Henan Medical University, Henan Province Hospital and Henan Tumor Hospital. Diagnosis of HCC was based on elevated serum alpha-fetoprotein (AFP >400ng/ml) together with a lesion in the liver detected by ultrasonography or computerized tomography. Of these HCC patients, 95 had a histopathological diagnosis of HCC. In all, 115 controls with non-hepatic disease were randomly selected from patients who were admitted to the same hospitals. Of these controls, 21, 62 and 32 suffered from diseases which needed treatment in the Divisions of Surgery, Internal Medicine and Cancer, respectively. Every control patient was definitely diagnosed as suffering from a disease which was not associated with hepatic disease. We selected controls so that distribution by sex and age was as similar as possible to that of cases. The characteristics of 152 HCC patients and 115 control patients are shown in Table 1. The demographic characteristics of the two groups were comparable. There were

Table 1 Demographic characteristics of hepatocellular carcinoma (HCC) patients and non-hepatic disease controls

Characteristics	HCC (N = 152)	Controls (N = 115)	P
Sex			
Male	136 (89.5%)	99 (86.1%)	
Female	16 (10.5%)	16 (13.9%)	>0.05 ^a
Age (years)			
Mean age	51.9 ± 12.5	52.5 ± 12.7	>0.05 ^b
Age range	18–88	17–86	
Education			
No schooling	14 (9.2%)	17 (14.8%)	
Elementary school	78 (51.3%)	54 (47.0%)	
Middle school	43 (28.3%)	32 (27.8%)	
High school	18 (11.8%)	12 (10.4%)	>0.05 ^a
Occupation			
Peasant	65 (42.8%)	48 (41.7%)	
Other	87 (57.2%)	67 (58.3%)	>0.05 ^a

^a χ^2 test.

^b Student's *t* test.

136 male patients and 16 female patients in the case group (mean age: 51.9 ± 12.5 years; age range: 18–88 years). There were 99 male patients and 16 female patients in the control group (mean age: 52.5 ± 12.7; age range: 17–86 years). In further analysis, a 1:1 pair-matched case-control study was performed. Of 152 HCC patients, 113 were randomly selected to be pair-matched by sex and age (± 5 years) to the controls with non-hepatic disease.

All the cases and controls were interviewed during hospitalization by two specially trained interviewers using a standard questionnaire which gathered information on demographic characteristics, past medical history, family history of liver disease and malignant neoplasm, history of alcohol drinking and cigarette smoking, dietary history (peanut, peanut oil, corn and vegetable consumption), history of contact with toxic substances (chemical or pesticide exposure), history of blood transfusion and psychosocial features.

All the blood specimens collected were aliquoted and stored at -70°C until tested. All sera were tested for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), antibody to HBsAg (anti-HBs), hepatitis B virus DNA (HBV-DNA) and antibody to hepatitis C virus (anti-HCV). The HBsAg and anti-HBs were tested by radioimmunoassay (RIA), and anti-HBc and anti-HCV by enzyme immunoassay (EIA), HBV-DNA was detected by using polymerase chain reaction (PCR). We also carried out reverse transcription polymerase chain reaction (RT-PCR) for the detection of HCV-RNA to confirm HCV infection in these sera with anti-HCV positivity. Infection with HBV was defined as that at least one of four markers of HBsAg, anti-HBs, anti-HBc and HBV-DNA was positive.

The odds ratio (OR) and 95% CI for the risk factors of HCC were calculated by logistic regression model, controlling for possible confounding factors such as sex and age, using the SAS statistical package. Multivariate analysis was done on the basis of the univariate analysis. The rates and ratios of this study were compared by using the χ^2 test. Probability values $P \leq 0.05$ were considered statistically significant.

Table 2 The prevalence of hepatitis B surface antigen (HBsAg), hepatitis B virus DNA (HBV-DNA) and antibody to hepatitis C virus (anti-HCV) in hepatocellular carcinoma (HCC) patients and controls with non-hepatic disease

HBV or HCV status	HCC (N = 152)		Controls (N = 115)		χ^2	P
	+	%	+	%		
HBsAg (+)	96	63.2	6	5.2	92.75	<0.001
HBV-DNA (+)	37	24.3	2	1.7	26.71	<0.001
HBsAg(+) and HBV-DNA(+)	32	21.1	2	1.7	21.89	<0.001
HBsAg(+) or HBV-DNA(+)	101	66.4	6	5.2	101.82	<0.001
HBV (+)	116	76.3	11	9.6	116.52	<0.001
Anti-HCV (+)	17	11.2	4	3.5	5.34	<0.05
HBsAg(+) and Anti-HCV(+)	12	7.9	0	0.0	9.47	<0.01
HBsAg(+) or Anti-HCV(+)	101	66.4	10	8.7	89.56	<0.001
HBV(+) and Anti-HCV(+)	14	9.2	1	0.9	8.56	<0.01
HBV(+) or Anti-HCV(+)	119	78.3	14	12.2	114.04	<0.001

Results

The prevalence of HBsAg, HBV-DNA and anti-HCV in patients with HCC and controls

As shown in Table 2, the prevalence of anti-HCV was slightly higher in HCC patients (11.2%) compared with controls (3.5%). The prevalence of HBsAg, HBV-DNA and HBV infection in controls (5.2%, 1.7%, 9.6%) was significantly lower than that in HCC (63.2%, 24.3%, 76.3%). The rate of both HBsAg and/HBV-DNA positivity showed a significant difference between the two groups. The rate of both HBsAg and/or anti-HCV positivity or both HBV and/or anti-HCV positivity in the HCC group were 7.9%, 66.4% and 9.2%, 78.3%, respectively.

Analysis of HBV and HCV as risk factors for HCC

To assess the effect of interaction of HBV and HCV on the risk of developing HCC, we analysed the individual effect of these two viruses. The risk of developing HCC (Tables 3 and 4) was strongly associated with the presence of HBsAg (OR = 28.82;

95% CI: 11.18–78.78) or HBV infection (OR = 31.22; 95% CI: 13.86–72.15). However, there was no significant association between HCV infection and HCC (OR = 3.06; 95% CI: 0.46–20.24). Moreover, the risk of developing HCC increased significantly and showed an additive effect modification when both viral markers of HBV and HCV infection were considered (OR = 42.85). The OR values and the increased trend shown by Mantel-Haenszel's χ^2 test (Table 4) also suggested the additive effect of dual HBV and HCV infection on risk for HCC.

For the purpose of analysing HBV, HCV and other factors as risks for HCC, the unconditional logistic regression model for the multivariate analysis was done on the basis of the univariate analysis. These factors, including the history of liver disease (individual and family), alcohol drinking, cigarette smoking, dietary aflatoxin intake (corn, peanut and peanut oil consumption), history of contact with toxic substances (chemical or pesticide exposure), history of blood transfusion, psychosocial factors, HBV and HCV infection were selected into the model for multivariate analysis. The results showed that history of liver disease, history of alcohol drinking, dietary aflatoxin intake, psychosocial factors and HBV infection contributed to the development of HCC in Henan. In particular, the OR value of HBV infection was very high (OR = 44.58; 95% CI: 12.54–158.49). The detailed results related to the multivariate analysis will be addressed in a separate paper.

In a further analysis, a 1:1 pair-matched case-control study was performed. Of 152 HCC patients, 113 were randomly selected to be pair-matched by sex and age (± 5 years) to the controls. The results of univariate analysis of risk factors for HCC showed that HBsAg positivity (OR = 32.50; 95% CI: 13.20–80.00) and HBV infection (OR = 18.75; 95% CI: 9.04–38.89) presented significantly elevated HCC risks ($P < 0.001$), but there was no significant association between HCV infection and HCC (OR = 3.00; 95% CI: 0.88–10.27). The conditional logistic regression analysis for multivariate analysis also indicated that HBV infection was an important risk factor for HCC (OR = 30.17; 95% CI: 9.44–96.40). In this study, the results from 1:1 pair-matched case-control study were basically consistent with the results from the group-matched case-control study described earlier.

Table 3 Risk factor analysis of hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (anti-HCV) seropositivity in patients with hepatocellular carcinoma (HCC) and controls with non-hepatic disease

HBsAg status	Anti-HCV status	HCC (N = 152)	Controls (N = 115)	OR	95% CI	χ^2	P
-	-	51	105	1.00			
-	+	5	4	2.57	0.57–12.03	1.97	>0.05
+	-	84	6	28.82	11.18–78.78	84.41	<0.001
+	+	12	0	undefined		21.41	<0.001

Table 4 Risk factor analysis of hepatitis B virus (HBV) infection and antibody to hepatitis C virus (anti-HCV) seropositivity in patients with hepatocellular carcinoma (HCC) and controls with non-hepatic disease

HBV status	Anti-HCV status	HCC (N = 152)	Controls (N = 115)	OR	95% CI	χ^2	P
-	-	33	101				
-	+	3	3	3.06	0.46–20.24	1.92	>0.05
+	-	102	10	31.22	13.86–72.15	108.33	<0.001
+	+	14	1	42.85	5.52–906.34	29.29	<0.001

Discussion

This is the first reported case-control study of HCC in Henan, China. Our study provides further evidence that chronic HBV infection is strongly associated with the development of HCC among this population. In this study, seropositivity for HBsAg, HBV-DNA and anti-HCV in HCC were 63.2%, 24.3% and 11.2%, respectively which suggests that most HCC were the result of virus-related chronic liver disease.

A close relationship between the development of HCC and persistent HBV infection has been stressed in various areas of the world.⁶⁻⁸ Long before the discovery of HBV antigen, some researchers noted that chronic hepatitis and liver cirrhosis were the predetermining factors of HCC. Even with the earlier insensitive tests, it was repeatedly found that positive HBsAg serology was found in a much greater percentage of HCC cases than in controls. Improvement in the sensitivity of the serological test for HBV markers further confirmed the assumption that HBV infection is closely related to the development of HCC. From most case-control studies, the estimates of OR for HBsAg positivity are in the range 10-50 in countries at high or intermediate risk for HCC. The OR value from case-control studies in European populations appears to be lower. A study conducted in Italy⁹ reported an OR of 5.3. The OR value for HBsAg as a risk factor for HCC in populations in America,¹⁰ Japan,¹¹ Taiwan¹² and China¹³ were 17.3, 31, 26 and 48, respectively.

Hepatitis C virus infection may result in chronic hepatitis and liver cirrhosis. Increasing attention has recently been paid to the role of HCV in the pathogenesis of HCC. The prevalence of anti-HCV positives in HCC was found to be 62% in Spain,¹⁴ 65% in Italy,¹⁵ 29% in South Africa¹⁶ and 29% in the US.¹⁷ However, it was reported that 70-90% of Japanese HCC patients were positive for anti-HCV.^{4,18,19} In our study, the prevalence of anti-HCV was slightly higher among the HCC patients compared with the control population. The OR values of HBsAg positivity by itself and HBV infection when used to compare HCC patients and patients with non-hepatic disease indicate that HBsAg positivity or HBV infection is one of the most important risk factors for HCC. The OR values of HCV infection in our study were not significantly higher. This may be due to the small number of subjects. Moreover, the risk of developing HCC increased significantly and showed an additive effect modification when both viral markers of HBV and HCV infection were considered. Multivariate analysis also showed a similar conclusion about the role of HBV infection as a risk factor for development of HCC.

The mechanism of HCC development in association with the hepatitis virus has been well explained for HBV. It was shown that the HBV genome could be integrated into host chromosomal DNA at a high rate and activate cellular oncogenes. Recent studies have indicated that the HBV X gene induced liver tumours in transgenic mice and interacted directly with the p53 gene. Dysfunction of the p53 gene may promote the genomic instability.^{20,21} However, the mechanism of hepatocarcinogenesis of HCV remains unknown. Hepatitis C virus is a single-stranded RNA virus without reverse transcriptase, and there is no evidence that the HCV genome contains an oncogene or that it integrates into the host genome. Therefore, an 'insertion mutagenesis theory' or a 'promoter insertion model' cannot be applied. In order to answer these questions, an

animal model of HCV-related hepatocarcinogenesis would be helpful in establishing an aetiological association. Furthermore, it is shown that in 10-20% of all patients in our study, the aetiology of HCC was unknown. This suggests that there is some unknown viral agent or other factors. Our previous study found that there was a prevalence of 12.4% of hepatitis D virus (HDV) infection in Chinese HCC patients with HBsAg positivity. Recently, it was reported that a new viral agent, so-called hepatitis G virus (HGV), has been identified.^{22,23} The relationship between HDV, HGV infection and HCC development remains to be determined. Meanwhile, our multivariate analysis results also show that dietary aflatoxin intake, alcohol drinking and psychosocial factors might be related to the development of HCC in Henan region. At present, we cannot exclude the possibility that HDV, HGV infection and other environmental carcinogens may lead to HCC.

The significance of HCV infection as a risk factor for HCC warrants further evaluation because the number of subjects in our study was small. The precise role of dual infection in the development of HCC is unknown. The clinical manifestation of HCC tended to be more severe in patients with concurrent HBV and HCV infection than in patients with HBV infection alone. Superinfection of HCV in an HBsAg carrier can also cause episodic necroinflammation, which may explain the probably additive action of both viruses as risk factors for HCC. Our study has suggested that HCV and HBV infection are independent and probably additive risk factors for HCC. The findings of this study have also indicated that HBV has played the most important role in the development of HCC in Henan, China, which is similar to the results reported in other populations in China. We consider that control of HBV infection remains of key importance for eliminating HCC ultimately.

Acknowledgements

The authors thank Drs Eng M Tan, Edward KL Chan, Dunrui Wang and Weiguo Zhu (The Scripps Research Institute, La Jolla, CA) for their helpful discussion and suggestions. This study was supported in part by a grant from The Science and Technology Commission in Henan, PR of China.

References

- 1 Parkin D, Pisani P, Ferlay J. Estimates of the worldwide incidence of 18 major cancers in 1985. *Int J Cancer* 1993;**54**:594-606.
- 2 Simonetti RG, Camma C, Fiorello F, Politi F, Damico G, Pagliaro L. Hepatocellular carcinoma: a worldwide problem and the major risk factors. *Digest Dis Sci* 1991;**36**:962-72.
- 3 Yen FS, Shen KN. Epidemiology and early diagnosis of primary liver cancer in China. *Adv Cancer Res* 1986;**47**:297-329.
- 4 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990;**12**:671-75.
- 5 Shimotohno K. Hepatitis C virus as a causative agent of hepatocellular carcinoma. *Intervirology* 1995;**38**:162-69.
- 6 Beasley RP. Hepatitis B virus, the major etiology of hepatocellular carcinoma. *Cancer* 1988;**61**:1942-56.
- 7 Hadziyannis S, Tabor E, Kaklamani E, Tzonou A, Trichopoulos D. A case-control study of hepatitis B and C virus infections in the etiology of hepatocellular carcinoma. *Int J Cancer* 1995;**60**:627-31.

- ⁸ Vall Mayans M, Calvet X, Bruix J *et al*. Risk factors for hepatocellular carcinoma in Catalonia, Spain. *Int J Cancer* 1990;**46**:378–81.
- ⁹ Filipazzo MG, Aragoma E, Cottone M, Dardanoni G, Lanlarone F, Pagliaro L. Assessment of some risk factors for hepatocellular carcinoma: a case control study. *Stat Med* 1985;**4**:345–51.
- ¹⁰ Di Bisceglie AM, Order SE, Klein JL *et al*. The role of chronic virus hepatitis in hepatocellular carcinoma in the United States. *Am J Gastroenterol* 1991;**86**:335–38.
- ¹¹ Tanaka K, Hirohata T, Koga S, Sugimachi K, Kanematsu T, Nishino N. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res* 1991;**51**:2842–47.
- ¹² Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Hepatitis B and C virus infection as risk factors for liver cirrhosis and cirrhotic hepatocellular carcinoma: a case-control study. *Liver* 1994;**14**:98–102.
- ¹³ Zhang JY. The research progress of the relationship between hepatitis B, C infection and hepatocellular carcinoma. In: Zhang JY, Zheng PY, Zhang Y (eds). *Research and Practice in Viral Liver Diseases*. Chinese Science and Technology Press, 1995, pp.148–67.
- ¹⁴ Ruiz J, Sangro B, Cuende JI, Beloqui O, Riezu-Boj JI, Herrero JI. Hepatitis B and C viral infections in patients with hepatocellular carcinoma. *Hepatology* 1992;**16**:637–41.
- ¹⁵ Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989;**ii**:1006–09.
- ¹⁶ Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in Southern African blacks with hepatocellular carcinoma. *Lancet* 1990;**i**:873–74.
- ¹⁷ Yu MC, Tong MJ, Coursaget P, Ross RK, Govindarajan S, Henderson BE. Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. *J Natl Cancer Inst* 1990;**82**:1038–41.
- ¹⁸ Nishioka K, Watanabe J, Furuta S, Tanaka E, Iino S, Suzuki H. A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer* 1991;**67**:429–33.
- ¹⁹ Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995;**21**:650–55.
- ²⁰ Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991;**351**:317–20.
- ²¹ Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits P53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci USA* 1994;**91**:2230–34.
- ²² Linnen J, Wanges JJ, Zhang-keck, ZY. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996;**271**:505–08.
- ²³ James DG. Viral hepatitis G string. *Q J Med* 1996;**89**:159–60.