A case-control study of hepatocellular carcinoma in Hyogo Prefecture

Inoue, Yoshiki
Shiraki, Takashi
Doi, Hisaya
Yamanaka, Naoki
Sato, Shigeaki

Kobe J. Med. Sci. 46, 181／188, August 2000

A CASE-CONTROL STUDY OF HEPATOCELLULAR CARCINOMA IN HYOGO PREFECTURE

Yoshiki INOUE*, Takashi SHIRAKI*, Hisaya DOI**,
Naoki YAMANAKA***, and Shigeaki SATO*

*Department of Hygiene, and **Department of Microbiology
Kobe University School of Medicine

***First Department of Surgery,
Hyogo College of Medicine,
1-1, Mukogawa-cho, Nishinomiya, 663-8501, Japan

KEY WORDS

hepatocellular carcinoma; HCV antibody; HCV genotype; blood transfusion; alcohol

ABSTRACT

We conducted a case-control study to elucidate factors contributing to the development of hepatocellular carcinoma (HCC) in Hyogo Prefecture, Japan where its incidence is high. In this study, the incidence of hepatitis virus infection in HCC patients and lifestyle factors including dietary and drinking habits were investigated. One hundred and eight patients with HCC (89 males and 19 females) and 93 controls (63 males and 30 females) were enrolled. Hepatitis C virus (HCV) antibody was identified in 87% of cases of which HCV-RNA was further detected in more than 95%. Genotype distribution of HCV was similar to that reported for the Japanese infected with HCV. There was a positive correlation between HCC and history of blood transfusion (both male and female) and surgery (male). Alcohol consumption over a period of 30 years was higher in the HCC patients than in controls; but not significant. These data indicated that HCV infection is a primary contributing factor to the development of HCC in Hyogo Prefecture.

Received for publication : June 6, 2000

INTRODUCTION

Incidence of hepatocellular carcinoma (HCC) is high in Africa and south-east Asia.[2] It is also common in Japan and its incidence is increasing.[16] Hyogo Prefecture has higher than national average incidence and mortality and is exceptionally high within the Hanshin area and the area facing the Inland Sea.[9] A case study of primary liver cancer in the prefecture was conducted in 1987. At that time the HBs antigen-positive rate was 21% and 67% of cases had a history of hepatitis. However, that study was not a case-control and information of hepatitis C virus (HCV) infection was not obtained. After the discovery of HCV,[6] the important role of infection with this virus in the pathogenesis of HCC has been shown in many studies.[1,3,8] But the role of specific HCV genotypes in HCC development has not been determined.[5,21]

In this study we examined hepatitis viral infection in combination with lifestyle factors such as diet, alcohol consumption and smoking which might be related to development of HCC in Hyogo area.

MATERIALS AND METHODS

Subjects

Cases were newly diagnosed subjects recruited from 20 major hospitals located in Hyogo Prefecture in Japan during 1993-1996. All cases were HCC. Preliminary diagnosis was made by the abdominal ultra sonography, α-fetoprotein, protein induced by vitamin K absence or antagonist-Ⅱ (PIVKAⅡ) or abdominal computed tomography and confirmed in most cases by angiography. Pathological diagnosis was made with 66 cases (64%).

Controls were inpatients recruited in the same hospitals diagnosed as free of cancer, liver disease, HBV and HCV. Controls were patients with gastroduodenal ulcers (35 cases), diabetes (24 cases) hypertension (12 cases), colorectal polyps (2 cases) and bronchial asthma (1case). Hospital controls were chosen because they are more likely to be willing to cooperate than healthy individuals, thus minimizing bias to response and they are easily identified and readily available in sufficient numbers, thus minimizing cost and effort in the program. Controls were matched for sex and age (±5 years).

All the cases and controls were living in Hyogo Prefecture and the majority of them were born in the same prefecture (59%).

Methods

We obtained informed consent from all subjects. Each completed a self-administered questionnaire. The questionnaire was composed of items related to living history, medical history and lifestyle. Living history included places of birth and residence, marital state and occupation. Medical history included past and family histories and histories of blood transfusion and surgical operation. Items related to lifestyle included regularity of daily life, sleeping hours, dietary, smoking and drinking habits. For smoking and drinking habits, their amounts of consumption over the past 30 years were asked.

Information with regard to methods on diagnosis and blood chemistry including presence of HBs antigen and HCV antibody were obtained from the questionnaire filled in by the doctor in charge. The attending physician drew 5ml of blood from all subjects with heparin and an additional 5ml from HCC cases and the serum was separated. HCV genotype of HCV-antibody positive serum samples was analyzed either at the laboratory of one of the authors[7] as previously reported and at a contract laboratory.

Correlation between HCC and items related to living history, medical history and lifestyle was analyzed by the χ^2 methods.

RESULTS

Case results were obtained for 89 males and 19 females. Their average ages were 62.3 ± 8.2 for males and 66.1 ± 6.1 for females.

Hepatitis virus infection results

As shown in table Ⅰ, 87% of cases were positive for HCV antibody. The HBs antigen-positive rate was 9% and mixed infection with HCV and HBV was found in 4% of cases.

Table Ⅰ. Incidence of HCV antibody- or HBs antigen-positive cases.

	male	female	total
HCV antibody(+)	76/89 (85)[a]	18/19 (95)	94/108 (87)
HBs antigen(+)	9/86(10)	0/18 (0)	9/104 (9)
Mixed infection	4/86(5)	0/18 (0)	4/104 (4)

[a] positive cases / total subjects (%)

Of 83 HCV antibody-positive cases from whom blood samples were obtained, HCV-RNA was positive in 81 cases. Its genotype distribution is shown in Table Ⅱ, and it is similar to that reported for Japanese blood donors infected with HCV.[14]

Table Ⅱ. HCV-RNA genotype distribution.

1b	2a	2b	3b	unclassified	total
52[a]	19	4	4	2	81
(66)	(24)	(5)	(5)		

[a] Number of subjects (%)

Significant differences between cases and controls

Analysis was done with 74 cases (57 males and 17 females) and 74 controls (57 males and 17 females) matched for age and sex.

As shown in Table Ⅲ, history of blood transfusion in both sexes and history of operation in males were significantly correlated with HCC. Apparently higher ratios of family history of hepatitis C (11/51, 22%) and being single (4/57, 7%) in male cases were observed compared with controls (0/52 and 0/57, respectively) but statistical analysis on the significance was not able to be done.

Table Ⅲ. Items significantly correlated with HCC.

		cases	controls	P value
History of blood transfusion	male	21/50 (42)[a]	6/55 (1)	<0.05
	female	7/14 (50)	2/16 (13)	<0.05
History of operation	male	38/57 (67)	26/56 (46)	<0.05

[a] corresponding subjects / subjects who answered (%)

Total amount of alcohol consumption over the past 30 years as pure ethanol

was higher in cases (613 ℓ) than in controls (469 ℓ) in males, although the difference was not statistically significant. Other life styles factors such as diet, sleep, hobby and regularity of daily life were not correlated with HCC.

DISCUSSION

About 90% of cases of HCC in the present study were positive for HCV antibody and more than 95% of these were also positive for HCV-RNA. The HCV antibody-positive rate of people of 45 years or more, who took health checks at health centers in Hyogo Prefecture was 3.1% and among all HCV antibody-positive subjects, 60% were positive for HCV-RNA. The figures were similar to the national average.[23] HCV-RNA was present at a higher rate in HCC cases than in the general population. Thus the persistence of HCV-RNA may be a good marker for the development of HCC. Genotype distribution of HCV was almost the same as that reported for the non-HCC Japanese population infected with HCV.[12]

HBV infection was found to have decreased in this study compared with the study in 1987 in the same prefecture. It should be noted that there are many cases where HBV-DNA is present in the liver or blood although HBs antigen is negative[22] and these cases are also prone to develop HCC.[13] Cases of mixed infection with HCV and HBV[19] in the present study was 4%. Accordingly, a very strong correlation of HCV infection with HCC development [10,11,18,20] was confirmed also in the present study.

Correlations of history of blood transfusion and surgery with HCC shown in this study all indicate HCV transmission to cases of HCC.

There was also a possibility of higher alcohol consumption being correlated with development of HCC mostly due to HCV infection.[14] Some proposed mechanisms for ethanol-induced hepatocarcinogenesis include: higher replication of HCV;[17] induction of microsomal metabolizing enzymes (p450s) resulting in activation of procarcinogens; depression of the vitamin A level; and immunosuppression.[4] No other lifestyle factors were found to be of significance although smoking has been reported as of importance.[24]

In Japan infection with HCV and HBV is now much reduced because of the introduction of new preventive measures. However, there is still a significant number of subjects infected with HCV and HCC may be increasing. A very high positive rate for HCV-RNA in HCC cases compared with those positive for HCV

antibody in regular health checks suggests the importance of the test for HCV-RNA in cases of positive antibody regardless of the presence or absence of clinical symptoms of liver damage. And in cases positive for RNA, its eradication by interferon treatment is essential. Avoiding from drinking too much alcohol is another preventive measure.

ACKNOWLEDGEMENTS

We are indebted to the hospitals that participated in this case-control study and provided data on as well as blood samples of the study subjects. This work was supported in part by research grants from Hyogo Prefecture and the Shinryokukai, the alumni association of Kobe University School of Medicine.

REFERRENCES

1. Abe, K., Edamoto, Y., Park, Y.N., Nomura, A., Taltavull, T.C., Tani, M., and Thung, S.N. 1998. In situ detection of hepatitis B, C, and G virus nucleic acids in human hepatocellular carcinoma tissues from different geographic regions. Hepatology. 28: 568-572.
2. Beasley, R.P., Hwang, L.Y., Lin, C.C., and Chein, C.S. 1981. Hepatocellular carcinoma and hepatitis B virus, a prospective study of 22,707 men in Taiwan. Lancet. 2: 1129-1133.
3. Bruix, J., Barrera, J.M., Calvet, X., Ercilla, G., Costa, J., and Sancheztapias, J.M. 1989. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet. Ⅱ : 1004-1006.
4. Chao, A.J., Young, T.H., Tang, H.S., and Hsu, C.T. 1990. Alcohol and cancer. Ann. Rev. Pharmacol. Toxicol. 30: 219-249
5. Chayama, K., Tsubota, A., Arase, Y., Saito, S., Koida, I., Ikeda, K., Matsumoto, T., Kobayashi, M., Iwasaki, S., and Koyama, S. 1993. Genotypic subtyping of hepatitis C virus. J. Gastroenteol. Hepatol. 8: 150-156.
6. Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., and Houghton, M. 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 244: 359-362.
7. Doi, H., Apichartpiyakul, C., Ohba, K., Mizokami, M., and Hotta, H. 1996.

Hepatitis C virus (HCV) subtype prevalence in Chiang Mai, Thailand, and identification of novel subtypes of HCV major type 6. J. Clin. Microbiol. 34: 569-574.

8. Hasan, F., Jeffers, L.J., Medina, M.D., Reddy, K.R., Parker, T., and Schiff, E.R. 1990. Hepatitis C-associated hepatocellular carcinoma. Hepatology. 12: 589-591
9. Hisamiti, S., Tsuji, I., Nishino, Y., Sauvaget, C., and Sakka, M. 2000. Standardized mortality ratio at all municipalities from 1980 to 1994, in p198-203. Geographical distribution of cancer mortality in Japan. Tohoku Radiological Science Center.
10. Ikeda, K., Saito, S., Suzuki, Y., Kobayashi, M., Tsubota, A., Koida, I., Arase, Y., Fukuda, M., Chayama, K., Murashima, N., and Kumada, H. 1998. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: A prospective observation of 2215 patients. J. Hepatol. 28: 930-938.
11. Kasahara, A., Hayashi, N., Mochizuki, K., Takayanagi, M., Yoshioka, K., Kakumu, S., Iijima, A., Urushihara, A., Kiyosawa, K., Okuda, M., Hino, K., and Okita, K. 1998. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology. 27: 1394-1402.
12. Okamoto, H. and Mishiro, S. 1994. Genetic heterogeneity of hepatitis C virus. Intervirology. 37: 68-76
13. Omata, M. 1998. Treatment of chronic hepatitis B infection. N. Engl. J. Med. 339 : 114-115.
14. Oshita, M., Hayashi, N., Kasahara, A., Hagiwara, H., Mita, E., Naito, M., Katayama, K., Fusamoto, H., and Kamada, T. 1994. Increased serum hepatitis C virus RNA levels among alcoholic patients with chronic hepatitis C. Hepatology. 20: 1115-1120.
15. Pereta, F.P. 1996. Molecular epidemiology: insight into cancer susceptibility, risk assessment and prevention. J. Natl. Cancer. Inst. 88: 496-506.
16. Research Group for Population-based Cancer Registration in Japan. 1999. Cancer incidence in Japan. Gann Monograph on Cancer Research. 47: 83-87.
17. Sawada, M., Takada, A., Takase, S., and Takada, N. 1993. Effects of alcohol on the replication of hepatitis C virus. Alcohol. 28: 85-90.
18. Shiratori, Y., Shiina, S., Imamura, M., Kato, N., Kanai, F., Okudaira, T., Teratani, T., Tohgo, G., Toda, N., and Ohashi, M. 1995. Characteristic differences of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan.

Hepatology. 22: 1027-1032.

19. Shiratori, Y., Shiina, S., Zhang, P.Y., Ohno, E., Okudaira, Y., Payawal, D.A. Ono-Nita, S.K., Imamura, M., Kato, N., and Omata, M. 1997. Does dual infection by hepatitis B and C viruses play an important role in the pathogenesis of hepatocellular carcinoma in Japan? Cancer. 80. 2060-2067.
20. Takano, S., Yokosuka, O., Imazeki, F., Tagawa, M., and Omata, M. 1995. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: A prospective study of 251 patients. Hepatology. 21: 650-655.
21. Tanaka, K., Ikematu, H., Hirohata, T., and Kashiwagi, S. 1996. Hepatitis C virus infection and risk of hepatocellular carcinoma among Japanese; Possible role of type 1b(Ⅱ)infection. J. Natl. Cancer. Inst. 88: 742-746.
22. Yotuyanagi, H., Yasuda, K., Iino, S., Moriya, K., Shintani, Y., Fujie, H., Tsutsumi, T., Kimura, S., and Koike, K. 1998. Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology. 27: 1377-1382
23. Yano, M., Yatsuhashi, H., Inoue, O., Inokuchi, K., and Koga, M. 1993. Epidemiology and long term prognosis of hepatitis C virus infection in Japan. Gut: supplement S13-S16
24. Yu, H., Harris, R.E., Kabat, G.C., and Wynder, E.L. 1988. Cigarette smoking, alcohol consumption and primary liver cancer: a case-control study in the USA. Int. J. Cancer. 42: 325-328.