Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6547–6549, September 1990
Medical Sciences

Hepatitis C virus infection is associated with the development of hepatocellular carcinoma

(non-A, non-B hepatitis)

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Communicated by William J. Rutter, May 4, 1990

ABSTRACT **A possible causative role for the recently discovered hepatitis C virus (HCV) in the development of hepatocellular carcinoma (HCC) was investigated by assay of sera from HCC patients in Japan for antibodies to a recombinant HCV antigen and to hepatitis B virus (HBV) antigens. Among the 253 HCC patients examined, 156 (61.7%) had no serum markers of either a previous or a current HBV infection (group I), 46 (18.2%) were negative for HBV surface antigen but positive for anti-HBV surface and/or anti-HBV core antibody, indicating the occurrence of a previous, transient HBV infection (group II), and 51 (20.2%) were chronically infected HBV carriers as evidenced by positivity for HBV surface antigen (group III). The prevalence of HCV antibody in group I (68.6%) and II (58.7%) patients was significantly higher than for group III (3.9%) or in 148 additional patients with other (non-HCC) cancers (10.1%) ($P < 0.01$). Thus, there appears to be a strong association between HCV infection and the development of HCC, particularly in patients for which HBV infection cannot be implicated as a causative factor. The data also suggest an additional mode of transmission for HCV other than blood transfusion, since a history of blood transfusion was shown in only about 30% of the HCV antibody-positive HCC patients in groups I and II. A high prevalence of HCV antibody was also shown among patients with HCC whose disease was originally thought to be due to very high ethanol consumption.**

The development of specific serological tests for infection by hepatitis A virus (HAV) and hepatitis B virus (HBV) has revealed that a large proportion of hepatitis cases are not caused by either of these agents (1–3). The resultant diagnosis of exclusion, non-A, non-B hepatitis (NANBH), now accounts for 95% of all posttransfusion hepatitis and over one-third of sporadic, acute hepatitis cases in Japan. Although symptoms in the acute phase of this disease are generally less severe than with HAV or HBV infection, NANBH is much more likely to develop into a persistent, chronic state. Over 50% of posttransfusion NANBH cases become chronically infected versus less than 10% in the case of HBV infections; typically, no chronicity results from infection by HAV. It is also clearly established that chronic NANBH can develop into hepatic cirrhosis (4–6). Accumulated serological, pathological, epidemiological, and clinical evidence suggests a significant association of the HBV carrier state with hepatocellular carcinoma (HCC) (7, 8). In Japan, however, less than one-third of HCC patients are also chronic HBV carriers, and the number of surgically treated HCC cases with no serological markers of prior or current HBV infection has increased steadily in Japan during the last 10 years (9). This suggests another causative factor(s). It has been hypothesized that NANBH virus(es) might be the missing causative agent in HCC development (10–14).

The etiological agent(s) of NANBH has long been sought by many research groups (15, 16), and recently a NANBH agent, termed hepatitis C virus (HCV), was identified by molecular cloning and characterization of its RNA genome (17). By using a HCV antigen synthesized by recombinant DNA methods in yeast, a specific assay was developed for detection of circulating antibodies to HCV. Results obtained from application of this assay have shown that HCV is the major causative agent of transfusion-associated NANBH in the United States, Italy, and Japan, as well as of a large proportion (at least 60%) of sporadic cases of NANBH (18–20). The antibody assay can detect both HCV carriers and individuals who have had a previous, but clinically resolved, infection with HCV (20, 21). Using this assay, we have now examined the prevalence of HCV infection among HCC patients in Japan to determine whether an association exists between oncogenesis and HCV infection similar to that postulated previously for HBV.

MATERIALS AND METHODS

Patients and Sera. HCC cases were diagnosed clinically and pathologically at one of the authors' hospitals. All serum samples to be tested were collected at the time of initial HCC diagnosis. Additional serum samples from patients with miscellaneous cancers other than HCC (3 esophagus, 26 stomach, 22 colon, 11 rectum, 17 bile duct, 15 pancreas, 29 lung, 7 uterus, 5 ovary, 4 kidney, and 5 metastatic liver cancer) were also tested for HCV antibody.

HCV Antibody Assay. Radioimmunoassay methods for determination of HCV antibody were as previously described (18). In brief, a part of the HCV cDNA encoding nonstructural proteins (NS3/4) containing 363 viral amino acids was expressed in yeast as a fusion polypeptide with a human superoxide dismutase (SOD). This SOD/HCV fusion polypeptide (C100-3) was solubilized, purified, and coated onto the wells of microtiter plates. After addition of sample and incubation in order to capture any circulating HCV antibody

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Abbreviations: HCV, hepatitis C virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HBs, HBV surface; HBc, HBV core; HCC, hepatocellular carcinoma; NANBH, non-A, non-B hepatitis.

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in serum specimens, the immobilized antigen-bound antibody complexes were then detected using ^{125}I-labeled sheep antibody to human IgG (1 μCi/ml; 1 Ci = 37 GBq; Amersham). Samples exhibiting over 3550 cpm (i.e., at least 3 standard deviations above the mean of normal blood donors; $P < 0.01$) were considered to be positive (18). About 80% of HCC patients displayed antibodies to the C100 antigen employed in this assay (G. K., unpublished information).

HBV Serum Markers. Serum HBV surface (HBs) antigen was determined by reverse passive hemagglutination, anti-HBs antibody by passive hemagglutination, and antibody to HBV core (HBc) antigen by enzyme immunoassay.

RESULTS AND DISCUSSION

HCV Prevalence and HCC Occurrence. We examined serum samples from 253 clinically and pathologically diagnosed HCC patients (207 men and 46 women) for HCV antibody by the radioimmunoassay. The age of the patients ranged from 35 to 85 with a mean of 61 years. These patients were classified into three groups (Table 1) according to the status of serum markers for HBV infection as follows: group I (156 cases, 61.7%) contained patients whose sera were negative for any HBV-associated antigen or antibody; group II (46 cases, 18.2%) comprised individuals negative for HBs antigen but positive for anti-HBs antibody and/or anti-HBc antibody in low titers, indicating the occurrence of a previous, transient HBV infection; group III (51 cases, 20.2%) patients were positive for HBs antigen, indicative of an active, persistent HBV infection. HCV antibodies were detected with a significantly higher frequency in patients from group I (68.6%) and group II (58.7%) as compared with the HBV-carrier HCC patients from group III (3.9%) ($P < 0.01$; Table 2). A similar high prevalence of anti-HCV antibodies was observed in HCC patients from three different locations in Japan (Tokyo, Sendai, and Ehime). We also tested for HCV antibody in sera from 148 cases of miscellaneous cancers other than HCC and found that 10.1% of these patients had HCV antibodies (Table 2). In this group, 5 patients with metastatic liver cancer were included, all of whom were negative for the antibody. The HCC cases in groups I and II (not associated with the HBV carrier state) constituted ≈80% (202/253, 79.8%) of all HCC patients examined and, of these, two-thirds (136/202, 67.3%) tested positive for this HCV antibody. Thus, over half (136/253, 53.7%) of the HCC cases in this study exhibited an association with previous HCV infection.

Epidemiology of HCV Infection. The mean age of all patients in groups I and II was 62.8 ± 8.7 years (Table 1). The average ages of the patients who were positive for HCV antibody was 62.8 ± 8.8, 61.3 ± 7.8, and 61.5 for groups I, II, and III, respectively (Table 2). These latter age distributions did not differ from those of antibody-negative patients in groups I and II but were significantly higher than that of HBV-carrier HCC patients in group III (55.9 ± 9.6 years). The reason for this observation is unknown, but it might be related to the age at which infection is acquired: HBV carriers usually result from vertical transmission from mother to child, whereas HCV infection usually occurs later in life (22). In addition, the period required for development of HCC after infection may be different for the two viruses. Although there was a sex difference in the incidence of HCC in each of the three groups, we could not detect any sex difference in HCV antibody positivity.

Table 2. HCV antibody among HCC patients and non-HCC cancer patients

Diagnosis	HBV-status group*	HCV antibody-positive No.	Rate,† %	Male/female ratio (% male)	Age, years
HCC	I	109	68.6	86/23 (78.9)	62.8 ± 8.8
	II	27	58.7	26/1 (96.3)	61.3 ± 7.8
	III	2	3.9‡	1/1 (50.0)	61.5
	Total	138	54.5	113/25 (81.9)	62.7 ± 8.6
Non-HCC	I/II§	15	10.1	10/5 (66.7)	58.9 ± 20.1

*See text and Table 1 for characteristics of each group.
†Percentage of HCV antibody-positive individuals in each group.
‡$P < 0.01$.
§All were negative for HBs antigen.

About one-third of the HCV antibody-positive HCC patients who were negative for HBs antigen (groups I and II) had a history of blood transfusions. These occurred 7–40 years (mean, 22 years) before the development of HCC (Table 3); the two HBV-carrier HCC patients who were also positive for HCV antibody had previously received a blood transfusion (20 and 25 years before). The remaining HCV antibody-positive patients from groups I and II had no history of blood transfusion or previously recorded episodes of hepatitis.

The observed prevalence of HCV antibody (10.1%) among the 148 non-HCC cancer patients examined in this study was considerably higher than that of healthy blood donors in Japan, which is currently estimated to be 0.9–1.2% (20, 21). However, 7 of these 15 antibody-positive patients had received a blood transfusion and a further 2 individuals presented with either chronic active hepatitis or cirrhosis. These complicating and confounding risk factors may have contributed to the observed high prevalence of HCV antibody in those non-HCC patients examined when compared with the general population. There is no evidence implicating HCV infection in the development of other cancers.

HCV and Alcohol Consumption in HCC Patients. To assess a possible role of ethanol as a complicating or contributing factor in HCC, the 202 HCC patients comprising groups I and II were further divided into three subgroups according to the amount and duration of self-reported ethanol consumption (Table 4). A similar high prevalence of HCV antibody was found in all three subgroups, including the subgroup (A)

Table 1. Characteristics of HCC patients and non-HCC cancer patients

Group	HBV status/history* HBsAg	Anti-HBs	Anti-HBc	No. tested	Male/female ratio (% male)	Age, years
HCC						
I	−	−	−	156 (61.7%)	125/31 (80.1)	62.7 ± 8.7
II*	−	+/−	+/−	46 (18.2%)	41/5 (89.1)	62.7 ± 8.3
III	+	−	+†	51 (20.2%)	41/10 (80.4)	55.9 ± 9.6‡
Total				253 (100%)	207/46 (81.8)	61.0 ± 8.9
Non-HCC	−	NT	NT	148	95/53 (64.2)	61.1 ± 14.4

NT, not tested.
*These patients were negative for HBs antigen (HBsAg) but positive for anti-HBs and/or anti-HBc antibody in low titers (i.e., the % inhibition by enzyme immunoassay was <70% in 200-fold diluted serum).
† The % inhibition by enzyme immunoassay was >70% in 200-fold diluted serum.
‡$P < 0.01$.

Table 3. Blood transfusion history and HCV antibody status (+ or −) in HBs antigen-negative HCC patients (groups I and II) and non-HCC cancer patients

Blood transfusion	No. of patients					
	HCC group I		HCC group II		Non-HCC cancer	
	+	−	+	−	+	−
Yes	41 (38.3%)	14 (28.6%)	9 (33.3%)	9 (47.4%)	7 (46.7%)	20 (15.0%)
No	66 (61.7%)	35 (71.4%)	18 (66.7%)	10 (52.6%)	8 (53.3%)	113 (85.0%)
Total	107	49	27	19	15	133

Table 4. HCV antibody prevalence in relation to ethanol consumption in HCC patients negative for HBs antigen

Ethanol consumption	No. tested	No. (%) positive for HCV antibody
Subgroup A, very high	53	33 (62.3)
Subgroup B, high	39	23 (59.0)
Subgroup C, low or none	110	76 (69.1)
Total	202	132 (65.3)

Subgroup A consisted of patients drinking >900 ml of Japanese sake (equivalent to 108 g of ethanol) daily for >10 years. Subgroup B patients consumed a daily average of 500–900 ml daily for >5 years. Subgroup C patients consumed <500 ml or no ethanol at all. All three subgroups were derived from groups I and II described in the text based on self-reported patient histories of ethanol consumption.

comprising HCC patients whose disease was previously diagnosed both clinically and histopathologically as due to excessive alcohol consumption. These data imply a need for clinical reassessment of hepatic cirrhosis and HCC patients, since symptoms previously attributed solely to the cumulative effects of alcohol may actually be due to pathology associated with cryptic HCV infection.

Conclusions. More than 80% of HCC cases diagnosed in Japan are preceded clinically by cirrhosis and the remaining 20% by chronic hepatitis (23–25). All the cases described here were associated with these chronic liver diseases both clinically and histologically, and we have also detected a high prevalence of HCV antibody among other patients with chronic hepatitis and cirrhosis (unpublished data). The assay (particular antigen) employed in these studies detects HCV antibodies in ≈80% of patients with known HCV infection (G.K., unpublished information). Thus, the actual incidence of HCV in the HCC patients studied here is likely to be correspondingly higher (≈25%) than that reported. The high prevalence of HCV antibody among HCC patients with no HBV markers and the detection of the HCV genome (by reverse polymerase chain reaction methods) both in cancerous and pericancerous cirrhotic liver tissues (26) strongly suggest that this newly identified RNA virus is involved in the development of HCC. Furthermore, since about two-thirds of these HCV antibody-positive HCC patients had no history of blood transfusion, it appears that HCV is also transmitted efficiently by an unknown route(s) to cause sporadic cases of community-acquired hepatitis (18) that can subsequently develop into chronic hepatitis, cirrhosis, and HCC.

We thank W. J. Rutter, H. Shimojo, T. Yoneyama, H. Yoshikura, and T. G. Sanders for critical reviews of the manuscript and helpful discussions. We are grateful to T. Oda and K. Nishioka for helpful advice and encouragement; to W.-S. Lee, C. Kuo, Y. Kaminuma, F. Sakurai, and M. Hashimoto for technical assistance; and to T. Mizoguchi and P. Montes for preparing the manuscript. This study was supported in part by a Grant-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare and by a Grant-in-Aid for Overseas Scientific Research from the Ministry of Education, Science, and Culture, Japan.

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