Articles

Evidence for Hepatitis C Viral Infection in Patients With Primary Hepatocellular Carcinoma

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In testing for antibodies to the hepatitis C virus (anti-HCV) in 112 patients with primary hepatocellular carcinoma, 10 of 33 white patients (30%) and 15 of 79 Asian patients (19%) had a positive response to the antibody. The antibody profile to individual hepatitis C viral antigens and the presence of circulating hepatitis C viral RNA were determined in the 25 patients. The anti-HCV antibodies most frequently detected were toward the antigens from the core (C22) and NS3 regions. Serum hepatitis C viral RNA was present in 17 of the 25 patients (68%), and these patients tended to have serum levels of alanine and aspartate aminotransferases higher than those patients without viremia (136 ± 22 U per liter versus 64 ± 11 U per liter and 161 ± 26 U per liter versus 79 ± 14 U per liter, respectively, both $P < .05$).

Of the 15 Asian patients with hepatocellular carcinoma and anti-HCV, 4 (27%) had coexisting hepatitis B surface antigen (HBsAg) and 13 (87%) had antibodies to either hepatitis B core or surface antigen. Of the 10 white patients with anti-HCV, however, only 1 (10%) had hepatitis B virus antibodies ($P < .01$). Among 4 Asian patients with coexisting anti-HCV and HBsAg, 1 was found to have serum hepatitis B viral DNA and the other 3 had hepatitis C viral RNA. A history of blood transfusion was obtained from 12 of the 25 patients with anti-HCV (48%); 20 (80%) had coexisting cirrhosis. Our findings support the hypothesis that hepatitis C virus is an important etiologic agent in the development of primary hepatocellular carcinoma in both white and Asian patients in the United States.

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Primary hepatocellular carcinoma is a common malignant disease in many Asian and African countries. In these areas of the world, chronic infection with the hepatitis B virus (HBV) is one of the most important factors associated with the development of this rapidly fatal cancer.[1-3] After the hepatitis C virus (HCV) was cloned and the serologic test for detecting antibodies to HCV (anti-HCV) became available, the association of chronic HCV infection and hepatocellular carcinoma was more clearly defined.[4,5] Studies have indicated that in western countries and in Japan, where HBV infection is less endemic, the seropositive rate of antibody to the C100-3 antigen of HCV in patients with primary hepatocellular carcinoma ranged from 13% to 75%.[6-11] Also, the coexistence of anti-HCV with the hepatitis B surface antigen (HBsAg) and other antibodies related to HBV infection has been reported in these patients.[2,3,8-13] The role of these two coexisting hepatitis viruses in the pathogenesis of primary hepatoma remains unclear, however.

Although it is now generally accepted that the HCV is one of the major causes of acute and chronic hepatitis, a controversy remains as to whether chronic HCV infection is associated with the progression of life-threatening liver diseases. A recent study from the United States showed that after an average 18-year follow-up, the long-term mortality after transfusion-associated non-A, non-B hepatitis was no different when compared with that of the matched controls who received transfusion but in whom hepatitis C infection did not develop.[14] In that study, 11 of 568 patients with chronic non-A, non-B hepatitis died of cirrhosis, and 1 other patient died of hepatocellular carcinoma. These findings were similar to those in 984 subjects in the control group, of whom 10 died of cirrhosis and 2 died of primary hepatocellular carcinoma. Another report from Italy on patients who contracted hepatitis C after a transfusion showed that 4 of 135 patients died of cirrhosis and 1 died of hepatocellular carcinoma after a mean follow-up of 7.5 years.[15]

Our purpose in this report is to determine the frequency of HCV antibodies in a large series of Asian and white patients with primary hepatocellular carcinoma who were referred to our liver center. In these patients, HCV infection was determined by a newly developed C25 chimeric anti-HCV immunoassay. We also tested for

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ABBREVIATIONS USED IN TEXT

ALT = alanine aminotransferase
anti-HBc = antibody to hepatitis B core antigen
anti-HBs = antibody to HBsAg
anti-HCV = antibody to HCV
AST = aspartate aminotransferase
ELISA = enzyme-linked immunosorbent assay
HBsAg = hepatitis B surface antigen
HBV = hepatitis B virus
HCV = hepatitis C virus
OPD = O-L-phenylenediamine 2-hydrochloride
PCR = polymerase chain reaction
SOD = superoxide dismutase
TRIS = tris(hydroxymethyl)aminomethamine

antibodies directed toward individual, expressed, recombinant HCV structural and nonstructural antigens, determined the presence of coexisting past or present HBV infection, and used sensitive polymerase chain reaction (PCR) methods to detect the frequency of HCV RNA and HBV DNA in these patients.

Patients and Methods

From 1982 to 1992, the clinical data from 112 patients with primary hepatocellular carcinoma at the Liver Center, Huntington Memorial Hospital, Pasadena, California, were reviewed. Primary hepatocellular carcinoma was diagnosed either histologically or based on typical radiologic images (ultrasonography, computed tomography, celiac angiography, or magnetic resonance imaging), along with elevated serum α-fetoprotein levels (greater than the normal value of 20 μg per liter [20 ng per ml]). Underlying cirrhosis was diagnosed either histologically or clinically by the presence of either ascites or esophageal varices. Stored serum specimens were tested for HBsAg, antibody to HBsAg (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc) by using standard radioimmunoassay tests (Ausria II, Ausab and Corab, Abbott Laboratories, North Chicago, Illinois). The presence of anti-HCV, HCV RNA, and HBV DNA was determined by techniques described as follows.

Hepatitis C Virus Antigens, Multiantigen Enzyme Immunoassay, and Chimeric C25 Protein Enzyme-Linked Immunosorbent Assay

The expression of various HCV antigens and the procedures for multiantigen enzyme immunoassay and chimeric C25 enzyme-linked immunosorbent assay (ELISA) were done according to previously described methods.[16] Briefly, the C22 (119 aa), E1 (130 aa), and NS5 (942 aa) antigens, and chimeric C25 (858 aa) antigen containing segments from C22, C33C, and C100-3 were expressed as internal antigens within the yeast *Saccharomyces cerevisiae* as C-terminal fusions with human superoxide dismutase (SOD) using the method described previously for generating the C100-3 antigen.[4,5] The C33C antigen (266 aa) was expressed as an internal SOD fusion polypeptide in *Escherichia coli* using methods described earlier for the synthesis of the 5-1-1 antigen. The E2 antigen (257 aa) was expressed as a secretory protein in *Spodoptera frugiperda* insect cells by cloning the E2 gene segment downstream of the interleukin 2 signal sequence in a derivative of the baculovirus vector, PAC373.[17,18]

Recombinant HCV antigens and denatured SOD (control) were diluted to optimal concentrations in phosphate-buffered saline (pH 7.4) and coated on Immulon I plates (Dynatech, Chantilly, Virginia). The ELISA assays were carried out as follows: a test specimen was diluted 40-fold in sample diluent on the plate and incubated for an hour at 37°C and then washed. O-L-Phenylenediamine dihydrochloride (OPD) and hydrogen peroxide were added for horseradish peroxidase color development. The ELISA cutoff optic density values for antigens SOD, C25, C22, E1, E2, C33C, and NS5 were 0.40 plus the optic density from the corresponding antigen-negative control. The cutoff value for the C100-3 antigen was 0.45 optic density plus its negative control values. If the SOD antigen was reactive, then that specimen was considered to be nonreactive or indeterminate.

Hepatitis C Viral RNA

We established a highly sensitive reverse transcriptase-nested PCR procedure to detect the HCV RNA of all anti-HCV-positive patients with primary hepatocellular carcinoma by using the newly cloned recombinant *Thermus thermophilus* DNA polymerase.[19] Briefly, the HCV RNA was extracted from 100 μl of plasma by a single-step acid guanidinium thiocyanate-phenol-chloroform method[20] and converted into complementary DNA with recombinant *T thermophilus* DNA polymerase. The first PCR was subsequently amplified with the same enzyme.

The downstream primer of complementary DNA synthesis was mncr #2: 5′ CAT GGT GCT CGG TCT ACG AGA CCT CCC 3′ (antisense), and the upstream primer of the first PCR was L3: 5′ GTC TAG CCA TGG CGT TAG TAT-3′ (sense). The primer of the second PCR was mncr #4: 5′ CGC AAG CAC CCT ATC AGG CAG T-3′ (antisense). These primers were deduced from the highly conserved 5′ noncoding region of the HCV genome.[21,22] The primers were synthesized by using solid-phase phosphoramidite chemistry with an automated DAN Synthesizer (Applied Biosystems, models 380B, Foster City, California) and purified by reverse-phase chromatography (Poly-pak Cartridge, Glen Research Corporation, Sterling, Virginia).*

Two microliters of extracted sample RNA was added to 8 μl of a master mixture containing 1 × reverse transcriptase buffer (10 mmol per liter of tris[hydroxymethyl]aminomethamine [TRIS] hydrochloride, pH 8.3, and 90 mmol per liter of potassium chloride), 1 mmol per liter of manganese chloride, 200 μmol per liter of each diethylnitrophenyl thiophosphate, 0.75 μmol per liter of downstream primer mncr #2, and 2.5 U of rTth DNA polymerase (Perkin-Elmer Cetus, Norwalk, Connecticut). One cycle of 72°C was run for 15 minutes in a thermal cycler (Geneamp PCR system 9600, Perkin-Elmer Cetus). The mixture was kept on ice, and 40 μl of chelating

*Burt Goodman of the Amgen DNA technology group, Boulder, Colorado, assisted us in synthesizing the PCR primers.

buffer (5% glycerol, 10 mmol per liter of TRIS hydrochloride, pH 8.3, 100 mmol per liter of potassium chloride, 0.75 mmol per liter of edetate, 0.05% Tween 20) containing 1.5 mmol per liter of magnesium chloride and 0.15 μmol per liter of upstream primer L3 was added to each reaction mixture. After the initial denaturation step for 3 minutes at 94°C, 35 cycles of denaturation were carried out at 94°C for 15 seconds, and the mixture was annealed at 65°C for 15 seconds and amplified at 72°C for 15 seconds. A seven-minute longer extension step was performed after the last cycle to ensure complete polymerization. For the second round of PCR, 5 μl of the first PCR product was aliquoted to a 45-μl mixture containing 1 × PCR buffer (50 mmol per liter of potassium chloride, 10 mmol per liter of TRIS-hydrochloride, pH 8.3, 1.5 mmol per liter of magnesium chloride, and 0.01% of gelatin), 200 μmol per liter of each diethylnitrophenyl thiophosphate, 1.25 U of Taq DNA polymerase (Perkin-Elmer Cetus), and 0.2 μmol per liter of primer pairs of L3 and mncr #4 and amplified similarly. After the second amplification, 10 μl of the PCR product was analyzed by electrophoresis on 1.5% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light. The expected final PCR product is 233 base pairs. The following controls were carried out for each extraction and amplification step: HCV-positive control, normal control, and reagent control (without template). Throughout the whole process, the stringent contamination-control procedures were followed.[23]

Hepatitis B Viral DNA

We applied a nested-PCR procedure to amplify the HBV DNA. The primer pairs used in the procedure were from the highly conserved HBV DNA polymerase region described by Mack and Sninsky,[24] with minor modifications. These included MD03: 5′-CTC AAG CTT ATC ATC CAT ATA-3′, MD06: 5′-CTT GGA TCC TAT GGG AGT GG-3′, and SMD09: 5′-GGC CTC AGT CCG TTT CTC TTG-3′.

For HBV-DNA extraction, we used the microwave method described by Cheyrou and co-workers,[25] with minor modifications. Briefly, the microwave oven (Sharp 900 W, model R-5A83) was first allowed to warm for seven minutes at its maximum power. Thin-walled tubes (Perkin-Elmer Cetus) containing 10 μl of a patient's serum were irradiated for three minutes at 900 W. Then 45 μl of a master mixture (the same mixture used in the second HCV complementary DNA PCR amplification buffer, except that the primers were replaced by HBV primers MD03 and MD06) was added to each desiccated serum specimen. The PCR procedure was carried out by initial denaturation for three minutes at 94°C, followed by 45 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 15 seconds. After the last cycle of amplification, a seven-minute extension was done to ensure complete polymerization. For the second round of PCR, 5 μl of the first-round PCR product was subjected to another 35 cycles of amplification under the same procedures with fresh components and a new set of HBV primers (MD03 and SMD09).

After the second PCR amplification, 10 μl of the final PCR products was analyzed by electrophoresis on a composite agarose gel made of 1.5% Nusieve and 1.5% Sea-Kem (FMC Bioproducts, Rockland, Maine), stained with ethidium bromide, and photographed under ultraviolet light. The expected size of the final PCR product was 105 base pairs.

Data in the text were expressed as a mean plus or minus the standard error of the mean. Statistical analysis was done using the Mann-Whitney U test and Fisher's exact test.

Results

Among 112 (33 white and 79 Asian) patients with primary hepatocellular carcinoma, 25 (22%) had antibodies to a chimeric C25 enzyme immunoassay test. By this assay, which contained HCV antigens C22, C33C, and C100-3, HCV antibodies were detected in 10 (30%) of 33 white and 15 (19%) of 79 Asian patients. Of the 25 patients with hepatocellular carcinoma, 13 were women and 12 were men. The mean age of the 25 patients was 58 ± 3 years (range, 29 to 76 years). All 10 white patients were born in the United States. Of the 15 Asian patients, 3 each were born in Taiwan, China, and Vietnam, 2 in Burma, and 1 each in Japan, Hong Kong, Korea, and Indonesia. A history of blood transfusion was obtained from 7 of 10 white patients (70%) and 5 of 15 Asian patients (33%) 7 to 42 years (mean 29.2 ± 3.8 years) before the diagnosis of hepatocellular carcinoma ($P = .06$). The remaining 13 patients had no history of injecting drugs, and none were health care workers. Only three patients recalled a bout of jaundice 6, 17, and 26 years before the diagnosis of carcinoma. Two patients had a substantial alcohol intake (ethanol amount greater than 80 grams per day). Of 25 patients with hepatocellular carcinoma, 20 (80%) had clinical or histologic evidence of cirrhosis. The mean serum alanine aminotransferase (ALT) level in the 25 patients was 113 ± 17 U per liter, and the mean serum aspartate aminotransferase (AST) level was 134 ± 20 U per liter. The mean α-fetoprotein level was 15,165 ± 7,796 μg per liter (range, 2.8 to 183,915).

Analysis of the antibody response of the patients to multiple HCV recombinant antigens is shown in Table 1. Of the 25 patients, 24 (96%) had antibodies to C22 and C33C recombinant proteins, 18 (72%) had antibodies to NS5, and 13 (52%) had antibodies to C100-3 and to E1. Only 4 (16%) had antibodies to E2, and none reacted to SOD.

Hepatitis C viral RNA as analyzed by PCR was present in 17 of the 25 patients (68%) (Table 1). The detection of HCV RNA in these 17 patients had no relationship to either age, sex, race, blood transfusion history, or to their anti-HCV profile. Table 2 shows the serum levels of aminotransferases in patients with and without HCV RNA. The HCV-RNA-positive patients had significantly higher serum levels of ALT and AST than those without detectable HCV viremia (136 ± 22 versus 64 ± 11 U per liter

TABLE 1.—*Race, History of Blood Transfusion, Serum Levels of Aminotransferase, and Antibody Response to Hepatitis C Viral (HCV) Antigens and HCV RNA in 25 Patients With Primary Hepatocellular Carcinoma*

		Aminotransferase, U/liter		*Antibody Response to HCV Antigens*							
Patients, No.	*Race*	*Alanine**	*Aspartate†*	*C25*	*C22*	*C33C*	*NS5*	*C100-3*	*E1*	*E2*	*HCV RNA*
1	White‡	367	207	+	+	+	+	–	–	–	+
2	White‡	22	55	+	+	+	+	–	+	–	–
3	White‡	59	47	+	+	+	+	+	–	–	–
4	White‡	64	63	+	+	+	–	+	–	–	–
5	White‡	63	124	+	+	+	+	+	+	+	–
6	White‡	30	55	+	–	+	–	–	–	–	–
7	White‡	83	147	+	+	+	+	+	+	–	+
8	White	63	91	+	+	+	+	–	–	–	+
9	White	67	109	+	+	+	+	+	+	–	+
10	White	56	96	+	+	+	+	+	+	–	+
11	Asian	175	317	+	+	+	+	+	+	–	+
12	Asian	57	111	+	+	–	–	–	–	–	–
13	Asian‡	52	46	+	+	+	+	+	+	–	+
14	Asian	233	480	+	+	+	+	–	–	–	+
15	Asian‡	115	135	+	+	+	+	+	+	+	–
16	Asian	119	179	+	+	+	+	–	+	–	+
17	Asian‡	143	111	+	+	+	+	+	+	+	+
18	Asian	125	174	+	+	+	+	–	–	–	+
19	Asian	103	62	+	+	+	+	–	+	–	–
20	Asian	75	97	+	+	+	–	–	+	+	+
21	Asian‡	81	71	+	+	+	+	+	–	–	+
22	Asian	273	167	+	+	+	–	–	–	–	+
23	Asian	215	150	+	+	+	–	+	+	–	+
24	Asian	171	242	+	+	+	+	+	–	–	+
25	Asian‡	19	46	+	+	+	–	–	–	–	+

\+ = positive, – = negative

*Normal range, 5 to 35 U per liter. †Normal range, 10 to 40 U per liter. ‡Patients with a history of blood transfusion.

and 161 ± 26 versus 79 ± 14 U per liter, respectively, both $P = .02$). Serum ALT levels greater than 70 U per liter and AST levels greater than 80 U per liter (2 times the upper normal limit) were present in 12 (71%) and 14 (82%) of the 17 patients found to have HCV RNA, respectively, and were significantly higher than in 2 (25%) and 3 (38%) of 8 patients without HCV RNA (both $P = .04$).

Among 15 Asian patients with hepatoma, 4 (27%) had coexisting serum HBsAg antigenemia (patient numbers 11, 12, 13, and 14). Five HBsAg-negative Asian patients had anti-HBc only, and another 4 had both anti-HBs and anti-HBc (Table 3). This finding of past or present HBV infection in 13 (87%) of 15 Asian patients was significantly different from that of white patients, in whom only 1 of 10 (10%) had detectable antibodies to HBV, and this patient had anti-HBc only (patient number 8). Serum HBV DNA as determined by PCR was found in only one of the four Asian patients with coexisting HBsAg and anti-HCV (patient number 12). None of the other three HBsAg-positive patients or other patients with primary hepatocellular carcinoma with anti-HBc or anti-HBs had detectable HBV DNA.

Discussion

During a ten-year period from 1982 to 1992, 112 patients with primary hepatocellular carcinoma were referred to the Liver Center at Huntington Memorial Hospital in southern California. Our patients are multiethnic and from all socioeconomic tiers. A preliminary description of the hepatitis B and C serologic test results in Asian-American patients with hepatocellular carcinoma has been reported elsewhere.[26] Using a new chimeric C25 protein assay, we determined that 22% of our patients with hepatoma had antibodies to the HCV. If the first-generation C100-3 antigen test was used, 48% of the patients would not have been identified. Thus, the chimeric C25

TABLE 2.—*Serum Levels of Aminotransferase in Patients With Hepatitis C Viral (HCV) Antibodies to Primary Hepatocellular Carcinoma and With and Without HCV RNA*

	Patients		
Serum Aminotransferase Levels	*HCV RNA Positive (n = 17)*	*HCV RNA Negative (n = 8)*	*P Values*
Alanine, ± SEM*	136 ± 22	64 ± 11	.02†
Serum level > 70 U/liter, No. (%)	12 (71)	2 (25)	.04‡
Aspartate, ± SEM§	161 ± 26	79 ± 14	.02†
Serum level > 80 U/liter, No. (%)	14 (82)	3 (38)	.04‡

*Normal range, 5 to 35 U per liter. †Mann-Whitney U test.
‡Fisher's exact test. §Normal range, 10 to 40 U per liter.

TABLE 3.—*Hepatitis B Viral (HBV) Markers in 25 Patients With Primary Hepatocellular Carcinoma Having the Antibody to Hepatitis C Virus*

Patients	*Positive for HBsAg*	*Positive for Anti-HBc Only*	*No HBV Markers*
White (n = 10), No	0*	1*	9*
Asian American (n = 15), No ...	4†	9‡	2

anti-HBc = antibody to hepatitis core antigen, HBsAg = hepatitis B surface antigen

*$P < .01$ compared with Asian patients.
†Only 1 patient had HBV DNA.
‡None of the patients had HBV DNA; 4 had antibodies to HBsAg.

was highly sensitive for detecting HCV antibodies in our hepatoma patients when compared with the C100-3 assay alone.

Of our white patients, 30% had the anti-HCV antibody. A history of transfusion was obtained from 7 of the 10 white patients with hepatocellular carcinoma (70%). The mean time of the transfusion in these patients was 29 years before the diagnosis of carcinoma. Whether the length of time between transfusion and the development of cancer or this mode of transmission of HCV plays a rôle in the progression to cancer in patients chronically infected with HCV remains to be elucidated. A recent report from the United States on the follow-up of patients with posttransfusion non-A, non-B hepatitis showed that the few patients who died of cirrhosis and primary hepatocellular carcinoma were similar to a transfused control group in whom hepatitis did not develop.[14] Of note, the follow-up time in the former report was an average of 18 years, and additional time may be required for the development of carcinoma in these patients with chronic HCV infection.

Only 20% of our Asian-American patients with carcinoma had the anti-HCV antibody. With the exception of Japan, most Asian countries have a high HBV carrier rate, and the cases of hepatocellular cancer from these countries are mostly related to HBV infection.[2,3] In our anti-HCV-positive Asian patients with carcinoma, four also had HBsAg and nine others had antibodies to HBV. Coinfection of HBV and HCV in these patients has been reported in other countries,[8-13] and several case-control studies have suggested a synergistic effect of these two hepatitis viruses in the pathogenesis of primary hepatocellular carcinoma.[27-29] Whether past or present HBV infection coexisting with chronic HCV infection increases the risk for the development of carcinoma requires more investigation. In our four Asian patients with coexisting anti-HCV and HBsAg, one was positive for HBV DNA and the other three were positive for HCV RNA. The coexistence of HBV DNA and HCV RNA was not detected in the same patient, suggesting viral interference. This finding is consistent with that of other studies indicating that HCV may predominate over HBV in patients with chronic hepatitis coinfected with both viruses.[30-33] Only five of our anti-HCV-positive Asian patients with hepatocellular carcinoma gave a history of transfusion. The rest reported no risk factors. But previous needle contamination from health care facilities in their native countries and acupuncture remain possible routes for HCV transmission in these persons.

In our 25 patients with hepatocellular carcinoma, the anti-HCV response to multiple HCV antigens occurred most frequently to recombinant proteins from the core (C22) and NS3 (C33C) regions of the HCV genome, followed in decreasing frequency by the response to antigens derived from the NS5, NS4 (C100-3), and E1 regions. The antibody response to the expressed proteins from the E2 regions was not as frequent as to those from other regions. This may be explained in part either by the use of linear E2 epitopes rather than conformational epitopes in our assays or by the hypervariability of the HCV sequences located in the E2 regions.[34]

Although the PCR assay for HCV RNA is highly sensitive in detecting circulating HCV, the sensitivity and specificity of PCR differed among research laboratories,[35] and the reported positivity rates of HCV RNA measured by PCR in hepatoma patients has varied.[33,36-39] One report from France showed that only 4 of 19 anti-HCV-positive patients with hepatocellular carcinoma (21%) were HCV-RNA-positive by PCR.[38] Our results showed that 17 of 25 patients with carcinoma (68%) who had the antibody to HCV had HCV RNA. This finding was consistent with recent reports on Spanish and southern African black patients with hepatocellular carcinoma.[37,39] Thus, the absence of HCV RNA in eight of our anti-HCV-positive patients may be due to either the inability of our method to detect low copy numbers of viral particles or that patients with chronic hepatitis C may have intermittent viremia. It is also possible that these patients had recovered from HCV infection and the primary hepatocellular carcinoma had another cause.

Higher levels of serum ALT and AST were noted in our patients with HCV RNA than in those without detectable HCV RNA. This finding suggests that in our hepatoma patients, HCV viremia was associated with ongoing liver damage. This is consistent with previous reports that abnormal liver function was more commonly found in anti-HCV-positive blood donors and in patients with chronic hepatitis C and HCV viremia than in those without HCV viremia.[36,40,41]

The high anti-HCV seropositivity rate in patients with hepatocellular carcinoma reported in many epidemiologic studies indicates a close relationship between chronic HCV infection and the eventual progression to cancer. The development of hepatocellular carcinoma through chronic hepatitis C and cirrhosis has been noted to occur after acute hepatitis C infection.[42] The high rate of cirrhosis in our anti-HCV-positive hepatoma patients (80%) is consistent with that in previous reports[12,43-45] and supports the earlier conjecture. Our findings indicate that the hepatitis C virus is an important cause of hepatocellular carcinoma in white and Asian patients in the United States. Accordingly, patients with chronic hepatitis C should be screened for hepatocellular carcinoma on a regular basis using serum α-fetoprotein testing and ultrasonography in the hopes of detecting this cancer early. Surgical resection of small hepatomas appears to be the most effective form

of treatment because HCV infection has been reported to recur regularly in patients receiving transplantation for chronic hepatitis C infection.[46]

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