Gastroenterologia Japonica
Copyright © 1993 by The Japanese Society of Gastroenterology

Vol. 28, No. 4
Printed in Japan

High prevalence of hepatitis B and C viral markers in Japanese patients with hepatocellular carcinoma

Naomi TANAKA[1], Toshiya CHIBA[1], Yasushi MATSUZAKI[1], Toshiaki OSUGA[1], Tatsuya AIKAWA[2], and Keiji MITAMURA[3]

[1]Department of Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan; [2]Aikawa Hospital, Mito, Japan; and [3]Second Department of Internal Medicine, School of Medicine, Showa University, Tokyo, Japan

Summary: In order to assess the etiologic role of hepatitis C virus (HCV) as well as hepatitis B virus (HBV) in the etiology of HCC, we compared the prevalence of HCV-related antibodies (anti-C100-3, anti-CP9, anti-CP10) and HBV-related markers (HBsAg, anti-HBs, anti-HBc) in sera of patients with liver cirrhosis (LC) with (n=62) and without (n=54) hepatocellular carcinoma (HCC). In HBsAg-negative cases, at least one HCV-related marker (including HCV RNA) was detected in 92.3% (48/52) of HCC cases and in all of the 44 LC cases without HCC, with no significant difference between these two groups. In HBsAg-positive cases, the prevalence of either one of these HCV-related markers was 40.0% (4/10) both in patients with and without HCC, and there was no significant difference between these two groups. Moreover, in HBsAg-negative cases and 11 cases of positive HCV-related markers, the prevalence of anti-HBs and/or anti-HBc was significantly higher in LC patients with HCC (85.4%) than those without HCC (43.2%, $P<0.001$). These results show a high prevalence of hepatitis B and C viral markers in Japanese patients with HCC and further indicate that previous HBV infection is a risk factor in the occurrence of HCC in HBsAg-negative LC and LC with positive HCV-related markers. *Gastroenterol Jpn 1993;28:547-553.*

Key words: *Hepatitis B viral markers, Hepatitis C viral markers, Hepatocellular carcinoma, Liver cirrhosis.*

Introduction

Abundant epidemiologic and molecular biologic evidence have established that hepatitis B virus (HBV) is a major etiologic factor in the development of hepatocellular carcinoma (HCC), and direct linkage of HBV to carcinogenesis has also been revealed[1]. It has been suspected that chronic non-A, non-B virus (NANBV) infection may also lead to the development of HCC. In Japan, a significant increase in the incidence of HCC has been noted for the past 20 years despite a steady decline in the prevalence of hepatitis B surface antigen (HBsAg) among HCC patients, suggesting the importance of NANBV infection in the pathogenesis of HCC. Indeed, shortly after the identification of hepatitis C virus (HCV) by the group at Chiron Laboratories[2] and the development of the C100-3 antibody test kit[3], it was disclosed in Japan as well as in other countries that the majority (60-90%) of HCC patients are positive for HCV antibody[4-11].

However, no report has provided comparative data on the frequency of HBV and HCV viral markers including HCV core antibody and HCV RNA in HCC.

To assess the etiologic role of the two viruses in the origin of HCC, we compared the prevalence of various HBV and HCV viral markers in Japanese patients with liver cirrhosis (LC) with and without HCC.

Received October 10, 1992. Accepted January 22, 1993.
Address for correspondence: Prof. Toshiaki Osuga, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305, Japan.

Meterials and Methods

Patients

The records of patients with LC admitted to the Tsukuba University Hospital between January 1986 and March 1991 were reviewed. The diagnosis of LC was established if patients met either of the following criteria: histologic diagnosis by blind or laparoscopically guided liver biopsy or the presence of ascites combined with either the presence of esophageal varices or typical sonographic findings of the liver. The following were excluded: patients with a history of chronic alcoholic consumption (>80 g/day for 5 years or more), patients with autoimmune hepatitis (positive antinuclear antibody), and those with hemochromatosis or primary biliary cirrhosis. One hundred sixteen cases fulfilled the criteria for inclusion. Among these, 62 cases had HCC. The diagnosis of HCC was established either by a histologic diagnosis on ultrasonographically guided fine-needle aspiration biopsy or by an elevated serum alpha-fetoprotein value (>400 ng/ml) combined with positive results on at least one imaging study (ultrasonography, computed tomography, angiography). All serum samples were stored at −20°C until tested.

Serum HBV markers

HBsAg was detected by reverse passive hemagglutination or radioimmunoassay (RIA). Antibodies against HBsAg (anti-HBs) were measured by passive hemagglutination. Antibody against the core antigen of HBV (anti-HBc) was examined by enzyme immunoassay (EIA) or RIA. The titers for anti-HBc with more than a 70% inhibition at a 1:200 serum dilution were assessed as positive.

Serum HCV markers

All serum samples were tested for antibodies against C100-3 by the enzyme-linked immunosorbent assay (ELISA, Anti-C100-3, Ortho Diagnostic System Co., Ltd., Raritan, NJ, USA). Samples with optical density (OD) values less than the cut-off value (usually around 0.45) were considered negative. Positive samples were further subclassified into two groups: strongly positive (OD values more than twice the cut-off limit) and weakly positive (OD values less than twice the cut-off limit). Cases in which only anti-C100-3 was positive and cases in which anti-C100-3 was weakly positive were further examined by RIA (Dynabott Co., Ltd., Tokyo, Japan, anti-HCV RIA kit). All serum samples were tested for HCV core antibody as well as for anti-CP9 and anti-CP10 (by ELISA), which react with HCV core protein[12,13], respectively. Specimens with an OD cut-off value of more than 0.35 were assessed as positive. In cases in which none of the HCV antibodies (anti-C100-3, anti-CP9, and anti-CP10) were detected, HCV RNA was further tested by the polymerase chain reaction (PCR) method. Briefly, RNA was extracted from serum samples followed by synthesis of cDNA by transcriptase. As far as the primer is concerned, we carried out two stages of PCR, using the 5′-noncoding region of HCV sequence determined by Okamoto and colleagues[14]. When either one of the antibodies (anti-C100-3, anti-core) or HCV RNA was positive, the samples were considered infected by HCV.

Statistical analysis

Data concerning age, serum albumin, serum total bilirubin, ICG R15 and prothrombin time were statistically analyzed using the Mann-Whitney test (two-sided test). Other data were statistically analyzed by chi-square test.

Results

The mean age of the patients was 58.6 years and 68 were males (58%). Thirty-four patients (29.3%) had a history of blood transfusion. **Table 1** summarizes the clinical characteristics of the HBsAg-positive as well as HBsAg-negative LC patients with (LC-HCC) and without (LC-nHCC) HCC. The mean age of HBsAg-positive LC-HCC (57.6 years) did not differ significantly from that of HBsAg-positive LC-nHCC (53.7 years). Cases of HBsAg-negative LC-HCC (61.6 years) showed a significantly higher mean age than HBsAg-negative LC-nHCC (56.3 years, $P<0.005$). Patients with HBsAg-negative LC-HCC were predominantly males, whereas those with HBsAg-negative

Table 1. Clinical characteristics and laboratory findings of patients with liver cirrhosis with and without hepatocellular carcinoma

	HBsAg-positive		HBsAg-negative	
	LC with HCC	LC without HCC	LC with HCC	LC without HCC
No. of subjects	10	10	52	44
Age (years)	57.6±12.4	53.7± 7.9	61.6± 7.6*	56.3±10.1*
Sex (M/F)	9/1	7/3	35/17**	17/28**
Past history of blood transfusion	2 (20.0%)	2 (20.0%)	12 (23.1%)	18 (40.9%)
Serum albumin (g/dl)	3.8± 0.6	4.0± 0.5	3.7± 0.6	3.9± 0.6
Serum total bilirubin (mg/dl)	1.0± 0.6	1.4± 1.6	1.5± 1.7	1.1± 0.7
ICG R15 (%)	14.7± 8.3	20.7±12.9	28.7±16.4	23.4±13.5
Prothrombin time (%)	83.2±18.5	64.1±15.6	78.5±16.9	79.9±18.8

HBsAg, hepatitis B surface antigen; LC, liver cirrhosis; HCC, heptocellular carcinoma; M, males; F, females; ICG, indocyanine green. All values are mean±S.D. *P<0.005 (Mann-Whitney test, two-sided test), **P<0.005 (chi-square test).

Table 2. Prevalence of virus markers for hepatitis C virus (HCV) in patients with liver cirrhosis (LC) with and without hepatocellular carcinoma (HCC)

	anti-HCV				anti-HCV(+) and/or
patients	anti-C100-3	anti-CP9	anti-CP10	HCV RNA	HCV RNA(+)
HBsAg-positive					
LC with HCC	2/10 (20.0%)	2/10 (20.0%)	1/10 (10.0%)	0/6 (0.0%)	4/10 (40.0%)
LC without HCC	0/10 (0.0%)	2/10 (20.0%)	2/10 (20.0%)	0/6 (0.0%)	4/10 (40.0%)
HBsAg-negative					
LC with HCC	40/52 (76.9%)*	35/52 (67.3%)	39/52 (75.0%)	1/5 (20.0%)	48/52 (92.3%)
LC without HCC	41/44 (93.2%)*	35/44 (79.6%)	38/44 (86.4%)	-	44/44 (100.0%)

anti-C100-3, antibody against C100-3; anti-CP9, antibody against CP9; anti-CP10, antibody against CP10. *P<0.05 (chi-square test).

LC-nHCC were mostly females. Cases of HBsAg-negative LC-nHCC had the highest frequency of past blood transfusions, although the incidence did not differ significantly from that of the other groups. No significant difference was observed among these groups in liver function parameters, except that the mean prothrombin time of patients with HBsAg-positive LC-HCC (83.2%) was significantly higher than that of patients with HBsAg-positive LC-nHCC (64.1%, P<0.05).

Table 2 summarizes the results of the serologic testings of the three anti-HCV antibodies (anti-C100-3, anti-CP9, and anti-CP10) and HCV RNA in HBsAg-positive as well as negative LC patients, with and without HCC. In cases of HBsAg-positive LC, HCV-related antibodies were positive in 4 of 10 LC-HCC (40.0%) and in 4 of 10 LC-nHCC (40.0%). Twelve cases with negative results on all 3 anti-HCV antibody testings also gave negative results on HCV RNA. In HBsAg-negative cases, on the other hand, a strikingly high prevalence of HCV-related markers was obtained. The positivity rates of anti-C100-3, anti-CP9, and anti-CP10 were 76.9%, 67.3%, and 75.0%, respectively in LC-HCC cases, and 93.2%, 79.6%, 86.4%, respectively, in LC-nHCC cases. The positivity rate of anti-C100-3 was significantly higher in LC-nHCC cases compared to that in LC-HCC cases (P<0.05), whereas no-difference was observed, between these two groups concerning the prevalence of anti-CP9 and anti-CP10. In 11 LC-HCC cases and 8 LC-nHCC cases, the anti-C100-3 ELISA test gave positive results, whereas both anti-CP9 and anti-CP10 tests were negative. In these cases in which only anti-C100-3 was positive, anti-C100-3 was also examined by RIA. Six of the 11 LC-HCC

Table 3. Prevalence of antibodies for hepatitis B virus in patients with liver cirrhosis (LC) negative for HBsAg and positive for hepatitis C virus-related markers with and without heaptocellular carcinoma (HCC)

Patients	anti-HBs	anti-HBc	anti-HBs-positive and/or anti-HBc-positive
LC with HCC	25/48 (52.1%)*	38/48 (79.2%)**	41/48 (85.4%)***
LC without HCC	9/44 (20.5%)*	18/44 (40.9%)**	19/44 (43.2%)***

anti-HBs, antibody against HBsAg; anti-HBc, antibody against HB core antigen. *P<0.005 (chi-square test), **, ***P<0.001 (chi-square test).

cases (54.5%) as well as all 8 LC-nHCC cases (100%) were positive by RIA. Only 5 HBsAg-negative cases showed negative results on all 3 anti-HCV antibody tests. When HCV RNA was examined in these cases, only one case was positive. In summary, at least one of these HCV-related markers could be detected in 92 out of 96 cases (95.8%) with HBsAg-negative LC; in 48 out of 52 LC-HCC (92.3%) cases and in all of the 44 LC-nHCC cases (100%).

The prevalence of HBV-related antibodies in HBsAg-negative LC and LC with positive HCV-related markers with or without HCC was then examined (**Table 3**). In LC-HCC cases, remarkably high positivity rates of anti-HBs (52.1%) and anti-HBc (79.2%) were observed, and either anti-HBs or anti-HBc was positive in 41 of 48 cases (85.4%). In LC-nHCC cases, on the other hand, a significantly lower prevalence of anti-HBs (20.5%, $P<0.005$ compared to HCC cases), and anti-HBc (40.9%, $P<0.001$ compared to HCC cases) was observed. Thus, these anti-HBV antibodies (either anti-HBs or anti-HBc) were detected significantly more frequently in LC-HCC (85.4%) than in LC-nHCC (43.2%, $P<0.001$). All of the anti-HBc positive sera showed a low antibody titer of anti-HBc (inhibition rate of less than 90% at a 1:200 dilution).

Finally, the pattern of incidence of both anti-HCV and anti-HBV antibody in HBsAg-negative LC was studied (**Table 4**). Positive results for both anti-HBV antibodies and anti-HCV antibodies were observed in 41 of 52 LC-HCC cases (78.8%), which was significantly more frequent compared to 19 of 44 LC-nHCC cases (43.2%, $P<0.001$).

Discussion

The HCV is a single chain RNA virus of 10,000 base pairs and is composed of a structural region, which forms a core and envelope and a nonstructural region. Previous studies regarding the relationship between HCV and HCC have used anti-C100-3 antibody, which reacts against the NS3~NS4 region of non-structural protein. A dramatic increase in the mortality rate of HBsAg-negative HCC has been reported in Japan[15]. The incidence of HCV in HBsAg-negative HCC cases as assessed using anti-C100-3, as has been reported to be 76.2%[15] or 94.4%[16] in Japan. Similar positivity rates of anti-C100-3 in cases of HBsAg-negative HCC have been reported in Spain (75%)[7], Italy (65%)[8], and Taiwan (63%)[17]. However, a low linkage between HCC and HCV has been reported in France (25%)[18] and in the United States (29%)[19]. The mortality rates of HCC in both males and females, on the contrary, are 10 times higher in Japan than in the United States per 100,000 people per year[15]. A high carrier rate of anti-C100-3 is found among the older generation with HCC in Japan, whereas the peak carrier rate of anti-C100-3 is in the age range 30–39 and 40–49 in the United States. In other words, the interval from the HCV infection until the emergence of HCC is shorter in the United States. These differences may be related to differences in HCV subtypes. Moreover, the differences in the incidence of HBV infection and in drinking habits should also be considered. Also, in these reports only anti-C100-3 HCV antibody was quantified by ELISA. ELISA quantification using anti-C100-3 may have produced a false positive result, espe-

Table 4. The pattern of incidence of antibody to hepatitis B virus (anti-HBV) and antibody to hepatitis C virus (anti-HCV) in paitnets with liver cirrhosis (LC) negative for HBsAg with and without hepatocellular carcinoma (HCC)

		LC with HCC		LC without HCC	
anti-HBV	anti-HCV	NO.	%	No.	%
+	+	41	78.8	19	43.2
+	−	2	3.8	0	0
−	+	9	17.3	25	56.8

P=0.002 (chi-square test). All cases gave positive results on either anti-HBV or anti-HCV.

cially when old serum samples or sera from patients with autoimmune hepatitis were used[20].

To overcome these shortcomings, more comprehensive assay methods, using antibodies to the core region of HCV[12,21] or second generation assay system, which detects both core and NS antibody to HCV[22,23], have been developed. Therefore, to obtain the entire picture of HCV infection, we measured both different anti-HCV antibodies and HCV RNA in cases negative for either anti-C100-3 or core antibodies.

To examine these from the viewpoint of the etiology of HCC, we investigated the prevalence of both HBV- and HCV-related markers in patients with HBsAg-negative LC. We found a high incidence of HCV-related markers in HBsAg-negative LC with (92.3%) and without HCC (100%). Since the positivity rate of HCV-related markers did not differ between these two groups, it is suggested that although HCV plays a major role in causing LC, its direct linkage to HCC could not be determined. On the other hand, we measured HBV-related antibodies in all patients with HBsAg-negative LC to determine the association with HBV. Cases where either anti-HBs or anti-HBc were positive represented 85.4% of LC-HCC, and 43.2% of LC-nHCC, showing a significant difference. In cases in which only anti-HBc was positive, none demonstrated a high inhibition rate at a 1:200 serum dilution, which suggests persistent infection by HBV. In the present study, a past history of infection by HBV was found frequently in HCV-related HCC. This indicated the possibility that transient infection by HBV may have some role in the development of HCV-related HCC.

In NANB-HCC patients with positive HBV-related antibodies, a possible involvement of HBV in the carcinogenesis has been pointed out[24-26]. There are some reports of infection with the two viruses, which some investigators have suggested to increase the risk for HCC. These studies have examined HBV markers and anti-C100-3[8,19,27]. Thus, we additionally studied HCV core antibodies as well as HCV RNA, and found that there were more cases with the appearance of HCV-related markers and HBV-related antibodies in patients with HBs-negative HCC. From these findings, the possibility arose that HBV infection might be involved in part of the HCC cases with positive HCV-related markers in the carcinogenic process.

In cases showing both positive HCV-related markers and anti-HBc, the anti-HBc value was low, indicating a past history of infection by HBV. HBV DNA was detected in liver tissue[28-30] from HBsAg-negative HCC or in half of the anti-HBs positive serum by PCR method[31]. From these reports, there may be persistent infection detected in some of the patients with HBsAg-negative chronic liver disease, which might induce liver injury and hepatocarcinogenesis. It is impossible to explain the origin of chronic liver disease by only transient HBV infection, but we cannot ignore that its occurrence may cause hepatocarcinogenesis through a "hit and run" mechanism[32] with the existence of chronic liver disease by HCV as a common lesion.

In HBsAg-positive LC, the prevalence of HCV-related markers was 40.0% in patients with and without HCC. According to previous reports, the prevalence of HCV antibody (anti-C100-3) in HBsAg-positive HCC ranged widely, from 2.2% to

58%[20,22,27,33-35]. Our results were relatively high. With the coexistence of HBsAg and HCV-related markers, there was no significant difference in the prevalence of HCV-related markers between LC-HCC and LC-nHCC. Taking into consideration that cirrhosis can progress to HCC, our data suggest that coinfection with the two viruses plays an important role in the origin of HCC.

Data strongly suggestive of direct involvement of HBV in inducing HCC have recently been demonstrated by Kim and colleagues[1]. On the other hand, whether HCV is directly involved in inducing HCC remains unclear. No evidence indicates the existence of reverse transcriptase in flavivirus or RNA virus, and it is not integrated into host DNA. Further studies are required to clarify the mechanism of the HCV-related hepatocarcinogenesis. Our epidemiologic findings indicate that a previous HBV infection might be a risk factor for the occurrence of HCC in HCV-related LC. Concerning the mechanism of carcinogenesis in patients with HCV-related HCC, the interaction between HCV and HBV, and the possibility that HBV is involved in carcinogenesis were predicted, but remain to be further elucidated.

The authors thank Dr. Masato Abei, Dr. Tadashi Yoshida, Dr. Yoshimichi Chuganji and Mrs. Sawako Kikuchi for preparation of the manuscript.

References

1. Kim CM, Koike K, Saito I, et al. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991;351:317-320.
2. Choo Q-L, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a bloodborne non-A non-B viral hepatitis genome. Science 1989;244:359-362.
3. Kuo G, Choo Q-L, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A non-B hepatitis. Science 1989;244:362-364.
4. Katkov WN, Cody H, Evans AA, et al. The role of hepatitis C virus (HCV) in chronic liver disease. (abstr.) Hepatology 1989;10:644.
5. Jeffers L, deMedina M, Hasan F, et al. Hepatitis C (HCV) associated idiopathic chronic hepatitis and cryptogenic cirrhosis. (abstr.) Hepatology 1989;10:644.
6. Alberti A, Tremolada F, Bortolotti F, et al. Anti-HCV and liver disease in Italy in the last 10 years. (abstr.) Hepatology 1989;10:644.
7. Bruix J, Barrera JM, Calvet X, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989;ii:1004-1006.
8. Colombo M, Kuo G, Choo Q-L, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989;ii:1006-1008.
9. Nishioka K, Watanabe J, Furuta S, et al. Antibody to the hepatitis C virus in acute hepatitis and chronic liver disease in Japan. Liver 1991;11:65-70.
10. Iino S, Kurai K, Hino K, et al. Detection of anti-HCV in patients with various liver disease. Acts Hepat Jap 1989;30:1643. (in Japanese)
11. The Liver Cancer Study Group of Japan. Survey and follow up study of primary liver cancer in Japan Report 8. Acts Hepat Jap 1988;29:1619-1626.
12. Okamoto H, Munekata E, Tsuda F, et al. Enzyme-linked immunosorbent assay for antibodies against the capsid protein of hepatitis C virus with a synthetic oligopeptide. Japan J Exp Med 1990; 60:223-233.
13. Okamoto H, Tsuda F, Machida A, et al. Antibodies against synthetic oligopeptides deduced from the putative core gene for the diagnosis of hepatitis C virus infection. Hepatology 1992;15:180-186.
14. Okamoto H, Okada S, Sugiyama Y, et al. Detection of hepatitis C virus RNA by a two stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. Japan J Exp Med 1990;60:215-222.
15. Nishioka K, Watanabe J, Furuta S, et al. A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. Cancer 1991;67:429-433.
16. 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;12:671-675.
17. Chen D-S, Kuo GC, Sung JL, et al. Hepatitis C virus infection in an area hyperendemic for hepatitis B and CLD: The Taiwan experience. J Infect Dis 1990;162:817-822.
18. Ducreux M, Buffet C, Dussaix E, et al. Antibody to hepatitis C virus in hepatocellular carcinoma. Lancet 1990;335:301.
19. Yu MC, Tong MJ, Cousaget P, et al. 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;82:1038-1041.
20. Wong DC, Diwan AR, Rosen L, et al. Non-specificity of anti-HCV test for seroepidemiological analysis. Lancet 1990;336:750-751.
21. Chiba J, Ohba H, Matsuura Y, et al. Serodiagnosis of hepatitis C virus (HCV) infection with an HCV core protein molecularly expressed by a recombinant baculovirus. Proc Natl Acad Sci USA 1991;88:4641-4645.
22. van der Poel CL, Cuypers HTM, Reesink HW, et al. Confirmation of hepatitis C virus infection by new four-antigen recombinant immunoassay. Lancet 1991;337:317-319.
23. Mimms L, Vallari D, Ducharme L, et al. Specifity of anti-HCV ELISA assessed by reactivity to three immunodominant HCV regions. Lancet 1990;336:1590-1591.
24. Maupas P, Werner B, Larouze B, et al. Antibody to hepatitis-B core antigen in patients with primary hepatic carcinoma. Lancet 1975; ii:9-11.
25. Kubo Y, Okuda K, Hashimoto M, et al. Antibody to hepatitis B core antigen in patients with hepatocellular carcinoma. Gastroenterology 1977;72:1217-1220.
26. Okuda K, Nakashima T, Sakamoto K, et al. Hepatocellular carcinoma arising in noncirrhotic and highly cirrhotic livers: A comparative study of histopathology and frequency of hepatitis B markers. Cancer 1982;49:450-455.

27. Yuki N, Hayashi N, Kasahara A, et al. Hepatitis B virus markers and antibodies to hepatitis C virus in Japanese patients with hepatocellular carcinoma. Dig Dis Sci 1992;37:65-72.
28. Brechot C, Nalpas B, Courouce A-M, et al. Evidence that hepatitis B virus has a role in liver-cell carcinoma in alcoholic liver disease. N Engl J Med 1982;306:1384-1387.
29. Brechot C, Degos F, Lugassy C, et al. Hepatitis B virus DNA in patients with CLD and negative tests for hepatitis B surface antigen. N Engl J Med 1985;312:270-276.
30. Paterlini P, Gerken G, Nakajima E, et al. Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. N Egnl J Med 1990;323:80-85.
31. Kaneko S, Miller RH, Feinstone SM, et al. Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. Proc Natl Acad Sci USA 1989;86: 312-316.
32. Galloway DA, McDougall JK. The oncogenic potential of herpes simplex virus: Evidence for a "hit-and-run" mechanism. Nature 1983;302:21-24.
33. Sbolli G, Zanetti AR, Tanzi E, et al. Serum antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. J Med Virol 1990;30:230-232.
34. Jeng J-E, Tsai J-F. Hepatitis C virus antibody in hepatocellular carcinoma in Taiwan. J Med Virol 1991;34:74-77.
35. Simonetti RG, Cottone M, Craxi' A, et al. Prevalence of antibodies to hepatitis C virus in hepatocellular carcinoma. Lancet 1989;ii: 1338.