Prevalence and Clinical Significance of HGV/GBV-C Infection in Patients with Chronic Hepatitis B or C

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SUMMARY: Hepatitis G virus/GB virus-C (HGV/GBV-C) is a newly identified Flavivirus. Its clinical significance in chronic hepatitis B and C remains controversial. Infection with HGV/GBV-C was surveyed in 500 blood donors, 130 patients with chronic hepatitis B and 173 with hepatitis C, with chronic liver disease, cirrhosis, and/or hepatocellular carcinoma (HCC). HGV/GBV-C RNA was detected by reverse transcription-polymerase chain reaction. An antibody to HGV/GBV-C’s second envelope protein (anti-E2 Ab) was detected using an enzyme immunoassay. The prevalence of HGV/GBV-C RNA was 3.4% and the exposure rate 10.2% in blood donors. The prevalence of HGV/GBV-C RNA in patients with chronic hepatitis B and hepatitis C was 7.7 and 17.3%, respectively (P = 0.002). The prevalence of the HGV/GBV-C infection in hepatitis B carriers increased with the severity of chronic liver disease and risk of HCC. The age and duration of hepatitis B virus infection were the more important contributing factors. Clinical and virological characteristics were comparable between those with and without coinfection of HGV/GBV-C and hepatitis C. The seroconversion rate was high. Coinfection of HGV/GBV-C with hepatitis B or C does not affect disease severity, but accelerates the progression of chronic liver disease and the development of HCC.

INTRODUCTION

A flavi-like virus designated the hepatitis G virus (HGV) or GB virus-C (GBV-C) has been identified in two independent laboratories (1,2). Deinhardt et al. reported sera from a surgeon (GB) who induced acute hepatitis and hepatocellular injury in primates (3). GB’s serum propagated in tamarins, and the viral agents were cloned and characterized as GB viruses A and B. Simons et al. detected and partially sequenced a virus with a sequence similar to that of GB viruses A and B, and named it GB virus C. Linnen et al. reported two isolates of the HGV, GBV-C and HGV, that shared 96-97% of the amino acid sequence and 86% of the nucleotide (2). Therefore, GBV-C and HGV could be two different genotypes of the same virus.

The genomic organization of HGV/GBV-C is similar to that of the Flaviviridae family. Even though HGV/GBV-C and hepatitis C virus (HCV) share strong sequence homologies, there is too wide a divergence between the virus sequences for HGV/GBV-C to be classified as genotypes of HCV.

HGV/GBV-C is transfusion-transmissible and has a global distribution. It has high prevalence in high-risk groups (4-8), such as patients receiving hemodialysis, hemophiliacs, and intravenous drug users (9-12). HGV/GBV-C has been found in some patients with chronic hepatitis or fulminant hepatitis without any evidence for known hepatitis virus infection (13). However, there are contradictory reports about the liver tropicity of HGV/GBV-C (14,15).

HGV/GBV-C infection has a high rate of spontaneous remission which involves the disappearance of HGV/GBV-C RNA and the production of antibody to HGV/GBV-C’s second envelope protein (anti-E2 Ab) (16-22). Most patients positive for anti-E2 Ab were negative for HGV/GBV-C RNA, and vice versa, indicating an inverse correlation of these two viral markers. Anti-E2 Ab can be detected in the beginning of HGV/GBV-C infection recovery and coexists with HGV/GBV-C RNA. There may be a window period between the disappearance of HGV/GBV-C RNA and the appearance of anti-E2 Ab.

Taiwan is an endemic area of hepatitis B and C. Both hepatitis B virus (HBV) and HCV may cause chronic liver disease and hepatocellular carcinoma (HCC). A high prevalence of HGV/GBV-C infection in Taiwan has been reported (6,18). Although the damage induced by HGV/GBV-C infection to the liver does not seem to be severe, there are some cases with prolonged viremia (19,20).

To determine the contribution of HGV/GBV-C to chronic hepatitis pathogenesis and sequel, we investigated the presence of the HGV/GBV-C and anti-E2 Ab in different groups: volunteer blood donors, and patients with chronic hepatitis B and/or C who were diagnosed with chronic persistent hepatitis, chronic active hepatitis, liver cirrhosis, or/and HCC. Our goals were to examine the natural history of HGV/GBV-C infection in those individuals in order to elucidate the epidemiology of HGV/GBV-C, determine the clinical significance of HGV/GBV-C infection in chronic hepatitis, and determine the effect of coinfection with hepatitis viruses (B or C).

MATERIALS AND METHODS

Patients: A total of 303 patients with chronic hepatitis were divided into 2 groups according to disease etiology: 130
patients with chronic hepatitis B and 173 patients with chronic hepatitis C. Chronic hepatitis B was defined by a positive reaction for HBsAg [HBsAg(+)], and HCV infection was defined by a positive second-generation anti-HCV assay result [anti-HCV(+)]. Coinfection with HBV and HCV was defined as HBsAg(+) and anti-HCV(+), with or without HCV RNA(+). All chronic hepatitis patients received liver biopsy examination and the biopsy tissue was sent to the Pathology Department of Kaohsiung Medical University Hospital for examination. HCC was diagnosed by the pathologist, mostly based on high α-fetoprotein levels and angiography studies. A total of 500 serum samples of healthy volunteer blood donors were obtained from Kaohsiung Blood Center in 1996 as controls.

Detection of HGV/GBV-C RNA in serum: HGV RNA in the serum was detected by nested reverse transcription-polymerase chain reaction (RT-PCR) using primers targeting the 5’UTR. Briefly, the total RNA was extracted from 140 μL of serum sample. After the RT reaction (using the Moloney murine leukemia virus reverse transcriptase), the first 30 cycles of PCR were performed with primers 5grl (5´-GCGAGTTGGTTAGGT CGTAAATCCCGGTCA-3´) (each cycle: 94°C for 1 min, 62°C for 45 sec, and 72°C for 1 min), and the next 35 cycles were performed with primers 5gf2, (5´-TGGTAGCCACT ATAGGGTGCTG-3´) and 5grl (5´-GGTTGGTAGGTCG-3´) (each cycle: 94°C for 1 min, 62°C for 45 sec, and 72°C for 1 min). The PCR products were analyzed by electrophoresis on a 3% agarose gel. In each PCR assay, two negative controls and one positive control were tested in addition to the samples of interest.

Measurement of anti-E2 Ab in serum: Anti-E2 Ab titer was measured by enzyme-linked immunosorbent assay (ELISA) from Boehringer Mannheim GmbH, Heidelberg, Germany according to the manufacturer's instructions.

HCV antibody and HBsAg detection: HCV antibody and HBsAg were detected with commercially available ELISA kits (Abbott, Chicago, Ill., USA). Serum samples that were positive for anti-HCV (ELISA) and negative for HCV RNA were retested for HCV with a recombinant immunoblot assay (Chiron® RIBA® HCV 3.0 Strip Immunoblot Assay; Bayer, Emeryville, Calif., USA). Alanine aminotransferase (ALT) (normal upper limit of serum ALT = 25 IU/L) was measured on a multichannel autoanalyzer.

Detection of HCV RNA in serum and HCV genotyping: To detect serum HCV RNA, nested RT-PCR was performed using 5’UTR-specific primers as described previously. Assignment to the HCV genotypes 1a, 1b, 2a, 2b, or 3a was determined by amplification of the core region using genotype-specific primers, as described by Okamoto et al. (23).

Detection of HBV DNA: HBV DNA was detected and quantified in the serum with the Quantiplex HBV DNA kit (Bayer).

Statistical analyses: Statistical analysis was performed using the SPSS computer software package (v10.0; SPSS, Inc., Chicago, Ill., USA), with descriptive statistics, Student t test, chi-square test and Spearman’s correlation coefficient. Values were deemed statistically significant at P < 0.05.

RESULTS

HGV/GBV-C in chronic hepatitis: HGV/GBV-C RNA was detected in 10 of 130 patients with chronic hepatitis B (7.7%) and 30 of 173 patients with chronic hepatitis C (17.3%). Anti-E2 Ab was found in 9 (6.9%) and 32 (22%) patients with hepatitis B and C, respectively. The exposure rate to HGV/GBV-C was significantly higher in patients with hepatitis C than in patients with hepatitis B (37% versus 14.6%, P = 0.002).

Patients with HBV and HGV/GBV-C coinfection were older than those infected by HBV alone (48.90 ± 6.89 versus 40.11 ± 14.75, P = 0.003). The mean ALT value of patients with a simple HBV infection was higher than that of patients coinfected with HBV and HGV/GBV-C (138.57 ± 205.77 versus 67 ± 31.16, P = 0.001). Sex, age, history, blood transfusion, HBV DNA level, and hepatitis B e antigen

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical characteristics of HBV carriers with and without HGV/GBV-C infection</th>
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<tbody>
<tr>
<td>HGV RNA(+)(+)</td>
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<tr>
<td>No. (%)</td>
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<tr>
<td>SEX M/F</td>
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<tr>
<td>Age (mean ± SD)</td>
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<tr>
<td>BT HX (+)</td>
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<tr>
<td>ALT (mean ± SD)</td>
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<tr>
<td>HBV DNA (pg/mL)</td>
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<td>HBV Ag(+)</td>
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Chronic hepatitis

<table>
<thead>
<tr>
<th></th>
<th>CPH</th>
<th>CAH</th>
<th>Liver cirrhosis</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPH</td>
<td>1 (0.8%)</td>
<td>16 (12.3%)</td>
<td>2 (1.6%)</td>
<td>15 (11.5%)</td>
</tr>
<tr>
<td>CAH</td>
<td>1 (0.8%)</td>
<td>52 (40.0%)</td>
<td>4 (3.1%)</td>
<td>49 (37.7%)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>1 (0.8%)</td>
<td>24 (18.5%)</td>
<td>3 (2.3%)</td>
<td>22 (16.9%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>7 (5.4%)</td>
<td>28 (21.5%)</td>
<td>10 (7.7%)</td>
<td>25 (19.2%)</td>
</tr>
</tbody>
</table>

* P = 0.003, Significance; † odds ratio = 3 (95% CI)
‡ P = 0.002, Significance; ‡ odds ratio = 2.34 (95% CI)
§ P = 0.001, Significance
∥ P = 0.19, No significance
* P = 0.022, Significance (Spearman’s correlation) (+)
HGV exp (+) = HGV/GBV-C exposure positive, ongoing infection plus past infection (with HGV/GBV-C RNA positive and/or anti-E2 Ab positive).
HGV exp (-) = HGV/GBV-C exposure negative, without ongoing infection nor past infection (with HGV/GBV-C RNA negative and anti-E2 Ab negative).
BT HX, blood transfusion history; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis.
(HBeAg) positivity were comparable between patients with chronic hepatitis B with and without HGV/GBV-C (Table 1). The existence of HGV/GBV-C RNA seemed to accelerate the progression of chronic liver disease ($P = 0.002$, Spearman’s correlation) (Table 1). The risk of HCC development increased with the existence of HGV/GBV-C RNA and the exposure to HGV/GBV-C infection, ongoing infection or past infection (with HGV/GBV-C RNA positive and/or anti-E2 Ab positive) (Table 1).

Among the 173 patients with chronic hepatitis C, there were no significant differences in sex, age, blood transfusion, ALT value, and distribution of genotype between those with and without HGV/GBV-C infection. HGV/GBV-C infection had no influence on the severity of chronic liver disease and the development of HCC (Table 2). There was no relationship between HCV seroconversion (the disappearance of HCV RNA from the serum) and HGV/GBV-C viremia or anti-E2 Ab (Table 2). The rate of seroconversion in HGV/GBV-C infection was 47.4 and 53% in the HBV and HCV carriers, respectively.

The odds ratio for the presence of HGV/GBV-C RNA was 3 (95% CI) in HBV carriers and 1.1 (95% CI) in HCV carriers. The odds ratio estimate for HGV/GBV-C exposure was 2.34 (95% CI) and 0.62 (95% CI) for HBV and HCV carriers, respectively.

HGV/GBV-C infection in volunteer blood donors: HGV/GBV-C RNA was detected in 17 of 500 blood donors (3.4%) and anti-E2 Ab was detected in 34 donors (6.8%). The prevalence of HGV/GBV-C exposure, ongoing infection or past infection (with HGV/GBV-C RNA positive and/or anti-E2 Ab positive) was 10.2% (51 of 500 donors). The male/female ratio, mean age, and ALT value were comparable between the HGV/GBV-C RNA positive group and negative group. Donors who had HGV/GBV-C exposure were significantly older than those without HGV/GBV-C exposure (34.47 ± 11.19 versus 29.08 ± 9.41 years, $P = 0.02$). In the blood donors, the rate of seroconversion in HGV/GBV-C infection was 66.7%.

**DISCUSSION**

In the present study, HGV/GBV-C RNA was detected in 3.4% of volunteer blood donors and the exposure rate (ongoing infection plus past infection) was 10.1%. Thus, the prevalence of HGV/GBV-C infection in southern Taiwan (3.4%), where HBV and HCV infection are endemic, is higher than that reported for northern Taiwan (2.1%). This indicates that HGV/GBV-C may share the same routes of infection with HBV and HCV. A high rate of exposure to HGV/GBV-C indicates that, besides parenteral transmission, additional route(s) of viral transmission may exist. The patients with HGV/GBV-C infection were older than those without infection. There was also no difference in the mean ALT level between the patients with and without HGV/GBV-C infection, suggesting that HGV/GBV-C may not cause obvious hepatocellular injury.

Coinfection with HGV/GBV-C in patients with chronic hepatitis B and C has frequently been observed. In this study, 18% patients with HGV/GBV-C infection were HBV carriers and 55% of the patients were coinfected with HCV, suggesting that HGV/GBV-C and HCV may share the same transmission route.

Patients coinfected with HBV and HGV/GBV-C were older than those infected with HBV alone. However, there was no difference in age between HCV carriers with and without HGV/GBV-C infection. Most HBV carriers in Taiwan became infected with HBV during the perinatal period of their childhood, and superinfection with HGV/GBV-C occurred thereafter. However, in Taiwan, the prevalence of HCV infection increases with age. Thus, caution should be taken

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Table 2. Demographic, clinical, and viral characteristics of patients with chronic hepatitis C, infected and not infected with HGV/GBV-C

<table>
<thead>
<tr>
<th></th>
<th>HGV RNA (+)</th>
<th>HGV RNA (–)</th>
<th>HGV exp (+)</th>
<th>HGV exp (–)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>30 (17.3%)</td>
<td>143 (82.7%)</td>
<td>64 (37%)</td>
<td>109 (63%)</td>
</tr>
<tr>
<td>SEX M/F</td>
<td>24/6</td>
<td>85/58</td>
<td>45/19</td>
<td>64/45</td>
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<tr>
<td>Age (mean ± SD)</td>
<td>46.37 ± 15.13</td>
<td>49.28 ± 13.07</td>
<td>47.47 ± 13.49</td>
<td>49.54 ± 13.43</td>
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<tr>
<td>BT HX(+)</td>
<td>6 (3.5%)</td>
<td>33 (19.1%)</td>
<td>13 (7.5%)</td>
<td>26 (15%)</td>
</tr>
<tr>
<td>ALT value</td>
<td>81.17 ± 69.57</td>
<td>89.27 ± 98.97</td>
<td>97.23 ± 108.06</td>
<td>82.36 ± 85.44</td>
</tr>
<tr>
<td>HCV RNA (+)</td>
<td>22</td>
<td>102</td>
<td>48</td>
<td>76</td>
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<tr>
<td>RNA (–)</td>
<td>8</td>
<td>41</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>HCV genotype (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I b</td>
<td>15 (50%)</td>
<td>70 (48.9%)</td>
<td>25 (39%)</td>
<td>50 (45.9%)</td>
</tr>
<tr>
<td>II a</td>
<td>11 (36%)</td>
<td>63 (44%)</td>
<td>21 (32.8%)</td>
<td>40 (36.7%)</td>
</tr>
<tr>
<td>II b</td>
<td>3 (10%)</td>
<td>5 (3.5%)</td>
<td>8 (12.5%)</td>
<td>5 (4.6%)</td>
</tr>
<tr>
<td>1b + II a</td>
<td>0</td>
<td>0</td>
<td>3 (4.7%)</td>
<td>6 (5.5%)</td>
</tr>
<tr>
<td>1b + II b</td>
<td>0</td>
<td>3 (2.1%)</td>
<td>2 (1.6%)</td>
<td>3 (2.8%)</td>
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<tr>
<td>Unclassified</td>
<td>1 (3%)</td>
<td>2 (1.4%)</td>
<td>5 (7.8%)</td>
<td>5 (5.5%)</td>
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<tr>
<td>Chronic hepatitis</td>
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<tr>
<td>CPH</td>
<td>6 (3.5%)</td>
<td>26 (15.0%)</td>
<td>13 (8.1%)</td>
<td>19 (11.0%)</td>
</tr>
<tr>
<td>CAH</td>
<td>13 (7.5%)</td>
<td>73 (42.2%)</td>
<td>33 (19.6%)</td>
<td>53 (30.6%)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>6 (3.5%)</td>
<td>19 (11.0%)</td>
<td>10 (7.0%)</td>
<td>15 (8.7%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>5 (2.9%)$^{11}$</td>
<td>25 (14.5%)</td>
<td>8 (4.6%)$^{2}$</td>
<td>22 (12.7%)</td>
</tr>
</tbody>
</table>

No significance between infected and not infected groups in all parameters.

1): odds ratio=1.1 (95% CI)
2): odds ratio=0.62 (95% CI)

HGV exp (+), HGV/GBV-C exposure positive; HGV exp (–), HGV/GBV-C exposure negative.

Abbreviations are in Table 1.
in interpreting the effects of HGV/GBV-C infection in chronic hepatitis B and C. HGV/GBV-C infection in the HCV/HBV endemic area is not associated with the presence of HBsAg. For HBV-HGV/GBV-C coinfection, discordant results were found in the literature; HGV/GBV-C RNA prevalence in hepatitis B chronic infection may be low: 0/48 in Wang and Jin (survey in professional blood donors, Taiwan) (24) and 7/220 (3.2%) Kao et al. (survey in patients with asymptomatic hepatitis B carriage and with chronic liver disease, northern Taiwan) (25), or high in other studies: 7/27(9.7%) (patients with chronic viral hepatitis, USA) for Linnen et al. (2), 32/100 (32%) (patients with acute viral hepatitis, USA) for Alter et al. (26). A recent report showed that, in France, a high prevalence of HGV/GBV-C RNA correlated with chronic HBV carriers that were coinfected with the human immunodeficiency virus (27). This finding is consistent with the view that the spread of HGV/GBV-C in HBV/HCV endemic areas is different from the spread of HBV in Taiwan, which is predominantly transmitted vertically from carrier mother to newborn or horizontally from child to child (28-30). The difference in the HBV-HGV/GBV-C coinfection pattern among the Far East, USA and Western Europe may be attributed to differences in HBV transmission routes in those areas (30).

The fact that the blood transfusion history was similar in both HGV/GBV-C-infected and noninfected patients, and that HGV/GBV-C RNA was found in the absence of hepatitis B and C infection indicates that the HGV/GBV-C virus is capable of independent transmission. Vertical transmission has been reported, and interspousal transmission was also suggested (25,31-33), although it was not well characterized. HGV/GBV-C viremia seemed to accelerate the progression of chronic liver disease in HBV carriers. However, the patient’s age and the duration of HBV infection may be the important contributing factors. The fact that the ALT value was similar in all chronic hepatitis patients regardless of HGV/GBV-C infection suggests that HGV/GBV-C cannot cause additive hepatic injury in patients with chronic hepatitis B or C. This result is comparable with that previously reported (34,35).

In the present study, gender, age, ALT levels, and the pathological distribution of chronic hepatitis were comparable between HGV/GBV-C viremic and nonviremic patients with chronic hepatitis. Additionally, blood donors with HGV/GBV-C viremia exhibited normal ALT levels. These results confirm that HGV/GBV-C infection exerts a minimal pathogenic effect in the HCV/HBV endemic area, as observed in previous reports (10,36-40). Reports containing pathological analyses also reveal no statistical difference in portal and/or lobular inflammation between HGV/GBV-C-infected and noninfected individuals (41,42), implying that HGV/GBV-C causes little hepatocellular injury and has no influence on the progress of chronic liver disease.

Hepatitis viruses may interact with each other in a synergetic, antagonistic, or additive manner to modify the course of the disease. Coinfection with hepatitis viruses may modify the clinical features of liver disease, for example by enhancing HBsAg seroconversion, and even increasing HBsAg clearance in chronic hepatitis B carriers coinfected with HCV (43,44). HBV or HCV infection status was not related to anti-E2 seroconversion, suggesting the involvement of a different immune mechanism in the clearance of HGV/GBV-C, which may partly be due to the deficit of the hypervariable region in the E2/NS1 region (20,21). Although a study showed that HGV/GBV-C coinfection was related to a particular HCV genotype (45), our results did not reveal this relationship. HGV/GBV-C and HCV, both Flaviviridae RNA viruses, did not seem to interact with each other. We also showed that there was no significant difference in terms of HCV RNA positivity, HBeAg positivity, and HBV DNA levels between patients with a single HBV or HCV infection and patients with HBV + HGV or HCV + HGV dual infections. This result indicates that HGV/GBV-C coinfection has no suppressive effect on HBV and HCV and does not aggravate the clinical course of chronic hepatitis B or C.

A higher prevalence of HGV/GBV-C infection was found in patients with HCC (46). Another study supported the hypothesis of an association between HGV/GBV-C infection and HCC, but the causality of the association was unclear (47). In contrast, studies by Kao et al. and Lightfoot et al. showed that HGV/GBV-C infection did not increase the risk of HCC development (25,48).

In the present study, HGV/GBV-C superinfection seemed to increase the relative risk of HCC development in HBV carriers, but not in HCV carriers. The increasing prevalence of HGV/GBV-C RNA in HBV-infected patients is associated with aging in Taiwan, where HBV carriers acquire HBV infection in their perinatal or childhood period. Age itself may contribute to the increased risk of HCC development. Even though this cross-sectional study does not clearly reveal the correlations between HGV/GBV-C and HCC, the role of HGV/GBV-C in HCC etiology seems modest. Additional longitudinal studies that include more cases are needed to resolve this issue.

In summary, we found that HGV/GBV-C infection is common in volunteer blood donors and more prevalent in patients with chronic hepatitis B or C. Blood transfusion is an important route for transmission. HGV/GBV-C seemed to cause little hepatic injury and did not aggravate the clinical course of chronic hepatitis B and C in this cross-sectional study.

ACKNOWLEDGMENTS

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REFERENCES


