GB virus C infection: Clinical significance

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XW Meng, M Komatsu, S Ohshima, et al. GB virus C infection: Clinical significance. Can J Gastroenterol 1999;13(10):814-818. GB virus C (GBV-C) RNA positivity rates were examined in serum specimens from 231 patients with liver disease (23 patients with hepatitis B, 175 patients with hepatitis C, five patients with hepatitis B virus plus hepatitis C virus coinfection, and 28 patients with non-A, non-B, non-C hepatitis) to clarify the clinical significance of this virus. GBV-C RNA was detected in none of 12 patients with fulminant hepatitis, one of two patients with acute hepatitis positive for hepatitis B surface antigen and one of four patients with acute non-A, non-B, non-C hepatitis. Pathogenetic involvement of GBV-C was suspected in some patients in the latter group. Among patients with the non-B, non-C type of chronic disease, one of seven with cirrhosis (14%) and none with chronic hepatitis or hepatocellular carcinoma were GBV-C-positive. In chronic hepatitis C patients who had received interferon treatment, no difference was found in clinical findings, alanine aminotransferase (ALT) concentrations, histology or response to interferon between 11 patients who were GBV-C RNA-positive and 101 patients who were GBV-C RNA-negative. Moreover, changes in ALT after interferon therapy showed no relation to positivity for GBV-C RNA. On the basis of these findings, GBV-C appears to be an unlikely cause of initiation or progression of chronic hepatic diseases.

Key Words: *Clinical study; GB virus C; Hepatitis G virus; Interferon therapy*

Infection au virus GB-C : signification clinique

RÉSUMÉ : Les taux de positivité pour l'ARN du virus GB-C (GBV-C) ont été examinés dans des échantillons de sérum provenant de 231 patients atteints d'une maladie hépatique (23 patients avec une hépatite B, 175 patients avec une hépatite C, cinq patients avec une hépatite au virus B et une hépatite au virus C concomitante, et 28 patients avec une hépatite de type non A, non B, non C) pour éclaircir la signification clinique de ce virus. L'ARN de GBV-C n'a été décelé chez aucun des 12 patients atteints d'une hépatite fulminante, chez un des deux patients avec une hépatite aiguë positive pour l'antigène de surface de l'hépatite B et chez un des quatre patients atteints d'une hépatite aiguë non A, non B, non C. L'implication pathogénique de GBV-C a été suspectée chez certains des patients du dernier groupe. Parmi les patients atteints d'une maladie chronique de type non B, non C, un patient sur sept atteints de cirrhose (14 %) et aucun patient avec une hépatite chronique ou un hépatome n'étaient positifs pour GBV-C. Chez les patients atteints d'une hépatite C chronique et qui avaient reçu un traitement par interféron, aucune différence n'a été observée dans les résultats cliniques, dans les concentrations d'alanine-aminotransférase (ALT), dans l'histologie ou la réponse à l'interféron entre les 11 patients positifs pour l'ARN de GBV-C et les 101 patients négatifs pour l'ARN de GBV-C. De plus, les changements dans l'ALT après un traitement à l'interféron n'ont révélé aucun lien avec la positivité pour l'ARN de GBV-C. Sur la base de ces résultats, il semble peu probable que le virus GB-C est responsable de l'apparition ou de la progression des maladies hépatiques chroniques.

In 1995 and 1996, respectively, Simons et al (1) reported GB virus C (GBV-C) and Linnen et al (2) reported hepatitis G virus as new hepatitis viruses capable of causing non-A through -E hepatitis. These two viruses were concluded to be identical because they belong to the species *Flaviviridae* and show 86% homology at the nucleotide level and 95% at the amino acid level (3). This virus infects hu-

mans, inducing acute hepatic injury and sustaining infection. However, its clinical significance remains largely unclear.

To address this uncertainty, we examined rates of GBV-C RNA positivity in patients with fulminant hepatitis, acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

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TABLE 1
GB virus C RNA in sera from patients with acute liver disease

Disease	HBsAg-positive	HCV RNA-positive	HBsAg and HCV RNA-positive	Non-A through -C*	Total
Fulminant hepatitis	0/3	0	0/1	0/8	0/12
Acute hepatitis	1/2 (50%)	0/2	0	1/4 (25%)	2/8 (25%)
Total	1/5 (20%)	0/2	0/1	1/12 (8.3%)	2/20 (10%)

*Immunoglobulin M antihepatitis A-negative, hepatitis B surface antigen (HBsAg)-negative and hepatitis C virus (HCV) RNA-negative

PATIENTS AND METHODS

Patients: A total of 231 patients (151 males, 80 females; mean age 51.8 ± 13.9 years) with viral hepatitis were selected randomly. They were hospitalized in the authors' department between January 1987 and December 1996: 12 with fulminant hepatitis, eight with acute hepatitis, 127 with chronic hepatitis, 40 with liver cirrhosis and 44 with hepatocellular carcinoma. All patients with chronic hepatitis and 28 with cirrhosis were diagnosed histologically. Patients with fulminant hepatitis and acute hepatitis, and 12 patients with liver cirrhosis were diagnosed by blood chemistry tests and hepatic imaging (ultrasound, computed tomography or magnetic resonance imaging). Patients with hepatocellular carcinoma were diagnosed histologically or by hepatic imaging including angiography.

For interferon (IFN) therapy, IFN- α was used in six cases, and a combination of IFN- β and IFN- α was used in five others. In all cases, therapy continued for six months. The total IFN dose was 702 to 780 MU. The trial of IFN therapy conformed to the ethical guidelines of the Declaration of Helsinki; treated patients gave informed consent.

The effect of IFN therapy was evaluated virologically as follows. Patients in whom GBV-C RNA or hepatitis C (HCV) RNA was negative six months after cessation of IFN therapy were defined as having a sustained response; others were considered nonresponders. In a similar fashion, patients in whom alanine aminotransferase (ALT) levels were normal for more than six months after cessation of IFN therapy were defined as having a sustained response; all others were categorized as nonresponders.

Detection of GBV-C RNA: Serum RNA was extracted from 100 μ L serum using the Sepa Gene RV-R kit (Sanko, Tokyo, Japan). cDNA was synthesized from the RNA sample at 37°C for 1 h using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, Maryland) and a random primer. GBV-C RNA was detected by reverse transcription-nested polymerase chain reaction (PCR) with specific primers derived from the 5' untranslated subgenomic region (4,5). First round PCR was performed with the sense primer 5gf2: 5'-GGTTGGTAGGTCGTAAATCCCGGTCA-3' and the antisense primer 5gr6: 5'-GACATTGAAGGGCGTRGACCGTAC-3', for 35 cycles consisting of denaturation for 1 min at 94°C, annealing for 45 s at 55°C and extension for 1 min at 72°C. Second round PCR was performed with the sense primer 5gf3: 5'-TGGTAGCCACTA TAGGTGGGT-3', and the antisense primer 5gr4: 5'GCGA

CGTGGACCGTACRTGGG CGT-3', for 35 cycles under the same conditions as for first round PCR. Amplicons were analyzed by electrophoresis on 3% agarose gels stained with ethidium bromide. Nucleotide sequences in PCR-positive subjects were determined by the dideoxy chain termination method using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Division, Foster City, California).

In each case, serum was collected aseptically, immediately frozen at -80°C, stored and thawed before examination.

RESULTS

The incidence of GBV-C RNA in patients with acute hepatic disease is shown in Table 1. Among patients with fulminant hepatitis, GBV-C RNA was not found in the serum initially in any case, but the serum became positive after plasma exchange in one patient. Among patients with acute hepatitis, one of two positive for hepatitis B surface antigen (HbsAg) and one of four with non-A through -C hepatitis were positive for GBV-C RNA.

Frequency of GBV-C RNA among patients with chronic hepatic disease was 18% for HBsAg-positive chronic hepatitis patients, 20% for cirrhosis patients and 16.7% for hepatitis B patients. Among HCV RNA-positive cases, prevalence of GBV-C RNA was 9.8% for chronic hepatitis, 4% for cirrhosis, 11% for hepatocellular carcinoma and 9.3% for hepatitis C. Among non-B, non-C hepatitis patients, positive cases accounted for 6.3% (Table 2).

Positivity for GBV-C RNA in blood and clinical features of patients with chronic hepatitis C are shown in Table 3. With respect to sex, mean age, history of transfusion, ALT, histology and therapeutic effect of IFN against HCV, no significant difference was observed among the 11 patients positive for GBV-C RNA and the 101 negative patients.

Eleven patients with chronic hepatitis showing combined infection with HCV and GBV-C were followed for longer than one year after IFN treatment. GBV-C RNA, HCV RNA and ALT were determined serially over time to assess the effect of IFN therapy. In three patients in whom GBV-C RNA showed a sustained response, ALT demonstrated a sustained response in one and no response in two. In eight patients in whom GBV-C RNA did not respond, ALT demonstrated a sustained response in two and no response in six. Meanwhile, in three patients in whom HCV RNA showed a sustained response, ALT demonstrated a sustained

TABLE 2
GB virus C RNA in sera from patients with chronic liver disease

Disease	HBsAg-positive	HCV-positive	HBsAg and HCV RNA-positive	Non-A through -C*	Total
Chronic hepatitis	2/11 (18%)	11/112 (9.8%)	0/1	0/3	13/127 (10.2%)
Liver cirrhosis	1/5 (20%)	1/26 (4%)	0/2	1/7 (14%)	3/40 (7.5%)
Hepatocellular carcinoma	0/2	4/35 (11%)	1/1	0/6	5/44 (11.4%)
Total	3/18 (16.7%)	16/173 (9.3%)	1/4 (25%)	1/16 (6.3)	21/211 (10%)

*Immunoglobulin M antihepatitis A-negative, hepatitis B surface antigen (HBsAg)-negative and hepatitis C virus (HCV) RNA-negative

TABLE 3
Clinical profiles of chronic hepatitis patients with isolated hepatitis C virus (HCV) versus patients with HCV plus GB virus C (GBV-C) coinfection

Clinical data	Chronic hepatitis patients with	
	HCV + GBV-C coinfection (n=11)	HCV infection (n=101)
Age (years)	42±9.5	47±13
Male/female	7/4	59/42
Transfusion history	2 (18.2%)	16 (21%)
Alanine aminotransferase (U/L)	107±78	102±107
Histology		
Chronic persistent hepatitis		9.3%
Chronic aggressive hepatitis 2a	90.1%	71%
Chronic aggressive hepatitis 2b	9.9%	19.7%
HCV-related response to interferon		
Sustained response	3 (27.3%)	34 (33.7%)
No response	8 (72.7%)	67 (66.3%)

All values are not significant

response in all. In eight patients in whom HCV RNA showed no response, ALT demonstrated no response in all (Figure 1).

The course of a patient with non-A through -C acute hepatitis and GBV-C RNA positivity is summarized in Figure 2. The patient noted dark yellowish urine about one month before medical consultation. About 10 days later, cold-like symptoms commenced. Maximum serum levels of ALT reached 1200 U/L, normalizing about 12 weeks later. Total bilirubin level reached a maximum of 23 mg/dL, persisting for about three weeks and normalizing about 16 weeks later. GBV-C RNA remained detectable throughout the course.

DISCUSSION

In patients with chronic hepatic diseases, GBV-C frequently has been found to coinfect with HCV. Linnen et al (2) reported the incidence of coinfection with GBV-C in chronic hepatitis C to be 18.8%, Dawson et al (6) about 20% and Schleicher et al (7) 19%. In Japan, coinfection has been re-

ported to range from 3% to 10% (4,8,9). In the present study, the incidence of coinfection was 9.8%.

In 11 patients who were GBV-C RNA-positive and 101 who were GBV-C RNA-negative, among those infected with chronic hepatitis C, no difference was observed in clinical findings, ALT or liver histology. Moreover, changes in ALT after IFN therapy frequently coincided with the changes in HCV RNA positivity but correlated less with positivity for GBV-C RNA. Therefore, we believe that HCV played the important role in hepatocellular injury in cases with coinfection. Antigenomic GBV-C RNA has recently been detected in the liver, revealing that the virus had proliferated there. However, in that report, no clinicopathological difference was found between GBV-C RNA-positive and -negative cases of chronic hepatitis (10). Thus, involvement of the virus in chronic hepatic diseases was felt to be minimal, as we also concluded.

All hepatocellular carcinomas associated with serum GBV-C RNA positivity were also associated with positivity for HBV- or HCV-related markers. In hepatocellular carcinoma associated with chronic non-B, non-C hepatitis, we found no patient to be positive for GBV-C RNA; Linnen et al (2) reported similar findings. Recently, an immunoassay for antibodies against a protein molecule encoded by the envelope 2 segment of the GBV-C genome was developed. Detection of these antibodies seems to exclude the viral genome from serum (11). Our study detects the presence of GBV-C; we did not perform the immunoassay for antibodies.

Existing reports on hepatocellular injury by GBV-C can be divided into two groups – one suggesting that the virus induces serious hepatic disorders and the other group concluding that resulting hepatic injury is mild. The former view was first reported by Yoshida et al (12) with respect to fulminant hepatitis of unknown etiology, three of six such patients were GBV-C RNA-positive. However, Kuroki et al (13) suggested that GBV-C could not be considered the cause of fulminant hepatitis because, of seven patients with fulminant hepatitis studied, no patient's serum was GBV-C RNA-positive before plasma exchange. In the present correlative survey, GBV-C RNA was negative before plasma exchange in all eight patients with fulminant non-A through -C hepatitis. Only one patient became positive after plasma exchange. These results suggest that infection with GBV-C and onset of fulminant non-A through -C hepatitis show little correlation.

Figure 1 Efficacy of interferon (IFN) therapy against GB virus C (GBV-C) in chronic hepatitis patients with GBV-C and hepatitis C virus (HCV) coinfection. **Panel A** Serial determinations of alanine aminotransferase (ALT) in three patients with virologic sustained responses according to GBV-C RNA. **Panel B** ALT in eight patients who were virologically nonresponsive according to GBV-C RNA. Correlation is relatively poor between GBV-C virologic response and the changes in ALT after cessation of IFN therapy. **Panel C** Serial determinations of ALT in three patients showing a sustained virologic response with respect to HCV. **Panel D** Serial profile of ALT concentration in eight patients with nonresponding HCV RNA positivity. A good correlation is seen between HCV virologic response and changes in ALT after cessation of IFN therapy

Figure 2 Clinical course of a patient with acute hepatitis associated with GB virus C (GBV-C) RNA positivity. This patient showed relatively severe jaundice with complete biochemical resolution of hepatitis. However, GBV-C RNA was persistent. ALT Alanine aminotransferase; T.Bil Total bilirubin

Infection with GBV-C was found in one patient with acute non-A through -C hepatitis. Hepatitis D virus coinfects with hepatitis B virus, and hepatitis E virus is not present in Japan. These findings indicate that the above patient was considered to have acute non-A through -E hepatitis. The patient complained of severe symptoms and demonstrated marked jaundice. This patient seemed to be infected with GBV-C alone, but viral RNA was still detected after clinical hepatitis had resolved. Alter et al (14) studied 10 patients with acute hepatitis in whom GBV-C RNA was positive, reporting that total bilirubin concentrations showed no elevation and that any changes associated

with GBV-C were mild, unlike the apparent situation of the patient we report here.

GBV-C is classified into three types by molecular analysis: one found predominantly in Africa, another dominant in America and the third most prevalent in Asia. The strain infecting the patient reported here was identified as the Asian type (4). Heringlake et al (15) reported characteristic base-substitution mutations commonly seen in the NS 3 region in patients with fulminant hepatitis and GBV-C RNA positivity. Accordingly, further study of the relationship between acute hepatic disorders and genetic variation among viruses is necessary.

REFERENCES

1. Simons JN, Leary TP, Dawson GJ, et al. Isolation of novel virus-like sequences associated with human hepatitis. *Nat Med* 1995;1:564-9.
2. Linnen J, Wages J Jr, Zhang-Keck ZY, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996;271:505-8.
3. Leary TP, Muerhoff AS, Simons JN, et al. Sequence and genomic organization of GBV-C: a novel member of the *flaviviridae* associated with human non-A-E hepatitis. *J Med Virol* 1996;48:60-7.
4. Orito E, Mizokami M, Nakano T, et al. GB virus C/hepatitis G virus infection among Japanese patients with chronic liver diseases and blood donors. *Virus Res* 1996;46:89-93.
5. Meng XW, Komatsu M, Ohshima S, et al. Efficacy of interferon therapy to GBV-C/HGV in patients with GBV-C/HGV and HCV coinfection. *Hepatol Res* 1997;8:130-8.
6. Dawson GJ, Schlauder GG, Pilot-Matias TJ, et al. Prevalence studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction. *J Med Virol* 1996;50:97-103.
7. Schleicher S, Chaves RL, Dehmer T, Gregor M, Hess G, Flehmig B. Identification of GBV-C Hepatitis G RNA in chronic hepatitis C patients. *J Med Virol* 1996;50:71-4.
8. Sugai Y, Nakayama H, Fukuda M, et al. Infection with GB virus C in patients with chronic liver disease. *J Med Virol* 1997;51:175-81.
9. Zhang XH, Shinzawa H, Shao L, et al. Detection of hepatitis G virus RNA in patients with hepatitis B, hepatitis C, and non-A-E hepatitis by RT-PCR using multiple primer sets. *J Med Virol* 1997;52:385-90.
10. Madejon A, Fogeda M, Bartolome J, et al. GB virus C RNA in serum, liver, and peripheral blood mononuclear cells from patients with chronic hepatitis B, C, and D. *Gastroenterology* 1997;113:573-8.
11. Tacke M, Kiyosawa K, Stark K, et al. Detection of antibodies to a putative hepatitis G virus envelope protein. *Lancet* 1997;349:318-20.
12. Yoshihara M, Okamoto H, Mishihiro S. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. *Lancet* 1995;346:1131-2.
13. Kuroki T, Nishiguchi S, Tanaka M, Enomoto M, Kobayashi K. Does GBV-C cause fulminant hepatitis in Japan? *Lancet* 1996;347:908.
14. Alter HJ, Nakatsuji Y, Melpolder J, et al. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N Engl J Med* 1997;336:747-54.
15. Heringlake S, Osterkamp S, Trautwein C, et al. Association between fulminant hepatic failure and a strain of GBV virus C. *Lancet* 1996;348:1626-9.