Hepatitis G virus infection in chronic liver disease

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Abstract

Background—The hepatitis G virus (HGV), a recently identified member of the Flaviviridae family, can cause chronic infection in man but the role of this agent in chronic liver disease is poorly understood.

Aims—To evaluate the prevalence and meaning of HGV infection in a large series of patients with chronic liver disease.

Subjects—Two hundred volunteer blood donors, 179 patients with chronic hepatitis C, 111 with chronic hepatitis B, 104 with alcoholic liver disease, 136 with hepatocellular carcinoma, and 24 with cryptogenic chronic liver disease were studied.

Methods—HGV RNA was investigated in serum samples by reverse transcription and polymerase chain reaction amplification of the 5' non-coding region of HCV and hybridisation to a specific probe. The main features of HGV RNA seropositive and seronegative patients were compared.

Results—The prevalence of HGV infection was 3% in blood donors, 7% in chronic hepatitis C, 8% in chronic hepatitis B, 2% in alcoholic liver disease, 4% in hepatocellular carcinoma, and 8% in cryptogenic chronic liver disease. HGV infected patients tended to be younger than non-infected patients but no differences concerning sex, possible source of infection, clinical manifestations, biochemical and virological parameters, or severity of liver lesions were found.

Conclusions—The prevalence of HGV infection in chronic liver disease seems to be relatively low in our area. Infection with HGV does not seem to play a significant pathogenic role in patients with chronic liver disease related to chronic HBV or HCV infection or to increased alcohol consumption, or in those with cryptogenic chronic liver disease.

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Keywords: chronic liver disease; hepatitis G virus

The hepatitis G virus (HGV) is a recently identified member of the Flaviviridae family distantly related to hepatitis C virus (HCV).^{1,2} This virus can be transmitted efficiently by blood transfusion and by other parenteral mechanisms²⁻⁵ and transient and long lasting infections with HGV have been documented in man.^{2,4}

HGV RNA sequences have been detected in a high proportion of volunteer blood donors.^{2,6} HGV RNA positive donors may present with minor increases in serum alanine aminotrans-

ferase (ALT) but at least 50% of them do not present with any evidence of liver disease. On this basis, HGV does not appear to be highly pathogenic.

Infection with HGV has, however, also been detected in a high proportion of patients with idiopathic fulminant hepatitis,^{7,8} suggesting that HGV may be highly pathogenic in some cases. This possibility is still open to question since other studies have not disclosed a significant prevalence of HGV infection in this condition.^{9,10}

There is also controversy about the role of HGV infection in the pathogenesis of chronic liver disease. Preliminary surveys of patients with chronic liver disease showed that HGV is often detected in patients chronically infected with other hepatotropic viruses such as the hepatitis B virus (HBV) or HCV but more rarely in patients with cryptogenic liver disease.^{2,11,12} Some studies have however found a relatively high prevalence of HGV infection in patients with cryptogenic chronic hepatitis, suggesting that this virus may be important in this condition.^{13,14}

To elucidate further the pathogenic role of HGV in chronic liver disease we investigated the prevalence of HGV infection and its associated features in 554 patients with different chronic liver diseases including representative series of patients with alcoholic liver disease and hepatocellular carcinoma.

Patients and methods

PATIENTS

Five hundred and fifty four patients with chronic liver disease and 200 consecutive first donation volunteer blood donors were studied. According to clinical, serological, and histopathological features, patients were separated into the following categories.

Chronic hepatitis C

This group included 179 patients (109 male and 70 female; mean age 50 years, range 19-83). Chronic hepatitis C was assessed by sustained elevation of ALT, histologically proved chronic inflammation of the liver, and positive tests for anti-HCV antibodies and for HCV-RNA in serum. Other causes of chronic liver disease were excluded.

Chronic hepatitis B

This group included 111 patients (92 male and 19 female; mean age 41 years, range 14-69) with chronic hepatitis B virus infection, as assessed by long lasting hepatitis B surface (HBsAg) antigenaemia and elevated ALT. Liver biopsy was performed in 71 cases.

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Alcoholic patients

This group included 104 patients (70 male and 34 female; mean age 43 years, range 22–76) who had consumed more than 80 g of ethanol per day for at least five years. HBsAg and anti-HCV were negative in all cases. Liver biopsy showed minimal abnormalities or fatty infiltration in 43 (41%), fatty infiltration and fibrosis in 14 (13.5%), alcoholic hepatitis in 23 (22.1%), hepatic cirrhosis in 21 (20.2%), and chronic hepatitis in three (3%).

Hepatocellular carcinoma

This group included 136 patients (91 male and 45 female; mean age 64 years, range 40–93) with hepatocellular carcinoma and underlying cirrhosis. The diagnosis of hepatocellular carcinoma was based on data from imaging techniques—ultrasonography, computed tomography, and/or hepatic arteriography—and confirmed by histopathological and/or cytological examinations. The aetiology of pre-existing cirrhosis was known in 125 cases.

Cryptogenic chronic liver disease

This group included 24 patients (12 male and 12 female; mean age 45 years, range 19–75) years. All presented with abnormalities of liver function tests consisting mainly of elevation of aminotransferase serum levels 1.5 times above the upper normal limit lasting for at least one year. None were obese or alcoholic, and all denied use of potentially hepatotoxic drugs. All had negative tests for HBsAg, anti-HCV, HCV RNA, and autoantibodies (antinuclear, antismooth muscle, antimitochondrial, and antiliver and kidney microsomes). Serum levels of iron, ferritin, caeruloplasmin, and α_1 -antitrypsin were normal in all cases. Liver biopsy showed minimal changes in 10 cases, fatty liver in four, minimal cholestasis in two, chronic hepatitis in two, portal and periportal fibrosis in two, and micronodular cirrhosis in four.

Blood donors

Two hundred consecutive volunteer blood donors (114 male and 86 female; mean age 39 years, range 18–65) were studied at the time of their first donation.

SEROLOGICAL METHODS

HBsAg, anti-HCV, and anti-HDV were tested with commercially available enzyme linked immunosorbent assay (ELISA) kits. HBV-DNA in serum was investigated by polymerase chain reaction (PCR) amplification of HBV core sequences according to a simplified protocol reported previously.¹⁵

HCV RNA in serum was determined by reverse transcription (RT) and amplification by nested PCR of the 5' non-coding region (5'NCR) region of HCV, as described previously.¹⁶ HCV RNA serum levels were measured by a competitive PCR method recently developed in our laboratory.^{17, 18} HCV genotype was determined according to Okamoto *et al*¹⁹ with some modifications, as described previously.²⁰

HGV RNA in serum was determined as described previously.^{10, 18} In brief, RNA was

extracted from 140 μ l of serum using a commercially available kit (QiAmp, Qiagen, Hilden, Germany). Complementary DNA was synthesised from 15 μ l of extracted RNA by 60 minutes incubation at 37°C with 300 U of Moloney leukaemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, Maryland, USA), random primers, and 20 U of ribonuclease inhibitor in a final volume of 30 μ l of RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.01% gelatine, 200 mM each deoxynucleotide). Specific PCR amplification and hybridisation of the 5'NCR of the HGV genome was carried out using a commercial kit (Hepatitis G virus-Primer and capture probe set, Boehringer, Mannheim GmbH, Mannheim, Germany) according to the manufacturer's instructions. One positive and three negative controls were included in each run. The background signal of negative controls ranged between 0.084 and 0.113. Samples giving a signal five times above the mean background signal were considered positive. Results were accepted on agreement on repeated testing.

STATISTICAL ANALYSIS

Comparisons between groups were made by the χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney test or Student's *t* test when appropriate for quantitative variables. A *p* value less than 0.05 was considered significant.

Results

HGV INFECTION IN BLOOD DONORS

HGV RNA was detected in six of 200 blood donors (3%). Anti-HCV was positive in two (1%) and HBsAg in one (0.5%). HGV infected donors were younger than non-infected donors (27 (5) versus 39 (14) years, *p*=0.03). None of the HGV infected donors had past history or clinical evidence of liver disease. ALT values were similar in HGV infected (32 (8) IU/l) and non-infected donors (29 (14) IU/l). A minimal elevation of ALT (45 IU) was detected in one of six (17%) HGV infected and in 28 of 194 (14%) non-infected donors.

HGV INFECTION IN PATIENTS WITH CHRONIC HEPATITIS C

HGV RNA sequences were found in 12 of 179 patients (6.7%). Although the prevalence of HGV infection in this group was higher than in blood donors the difference did not reach statistical significance. No significant differences between HGV infected and non-infected patients were observed concerning the demographic features, the presumed source of infection, the biochemical or haematological abnormalities, the degree of severity of liver lesions, the infecting HCV genotype, or the level of HCV viraemia (table 1).

HGV INFECTION IN PATIENTS WITH CHRONIC HBV INFECTION

HGV RNA was detected in nine of 111 patients (8.1%). Although the prevalence of HGV infection in this group was higher than in blood donors the difference was not statisti-

Table 1 Baseline features of patients with chronic hepatitis C according to the presence or absence of HGV-RNA sequences in serum

Characteristics	HGV-RNA negative (n=167)	HGV-RNA positive (n=12)	p Value
Age (y)	50 (14)	49 (17)	NS
Sex (male/female)	102/65	7/5	NS
Presumed source of infection			NS
Blood transfusion	51 (30%)	3 (25%)	
Addiction to i.v. drugs	15 (9%)	2 (17%)	
Unknown	101 (61%)	7 (58%)	
WBC count ($\times 10^9/l$)	5.6 (1.8)	4.1 (2.3)	NS
Platelet count ($\times 10^9/l$)	152 (55)	125 (65)	NS
AST (IU/l)	92 (61)	95 (80)	NS
ALT (IU/l)	151 (115)	186 (151)	NS
γ -glutamyl transferase (IU/l)	52 (44)	32 (15)	NS
Bilirubin (mg/dl)	0.9 (1.01)	1.0 (0.7)	NS
Degree of histological severity			NS
Mild hepatitis	55 (33%)	2 (16%)	
Moderate hepatitis	50 (30%)	4 (33%)	
Severe hepatitis	18 (11%)	2 (16%)	
Cirrhosis	44 (26%)	4 (33%)	
Virological features			
Genotype 1b	131 (80%)	8 (67%)	NS
Other genotypes	33 (20%)	4 (33%)	NS
HCV viraemia (copies/ml $\times 10^6$)	0.418 (0.873)	1.105 (1.719)	NS

Quantitative variables are expressed as mean (SD). Categorical variables are expressed as n (%).

Table 2 Baseline features of patients with chronic HBV infection according to the presence or absence of HGV-RNA sequences in serum

Characteristics	HGV-RNA negative (n=102)	HGV-RNA positive (n=9)	p Value
Age (y)	41 (13)	36 (5)	NS
Sex (male/female)	86/16	6/3	NS
Presumed source of infection			NS
Blood transfusion	1 (1%)	1 (11%)	
Addiction to i.v. drugs	2 (2%)	1 (11%)	
Intrafamilial	19 (19%)	1 (11%)	
Male homosexuality	6 (5%)	4 (44%)	
Unknown	74 (67%)	2 (22%)	
WBC count ($\times 10^9/l$)	5.9 (1.8)	5.5 (1.7)	NS
Platelet count ($\times 10^9/l$)	156 (47)	163 (61)	NS
AST (IU/l)	70 (54)	63 (29)	NS
ALT (IU/l)	125 (118)	111 (91)	NS
γ -glutamyl transferase (IU/l)	45 (47)	28 (7)	NS
Bilirubin (mg/dl)	0.8 (0.3)	0.6 (0.4)	NS
Degree of histological activity			NS
Mild hepatitis	16 (23%)	2 (29%)	
Moderate hepatitis	22 (31%)	1 (14%)	
Severe hepatitis	22 (31%)	3 (43%)	
Liver cirrhosis	8 (11%)	1 (14%)	
Other virological markers			NS
HBV-DNA positive	64 (63%)	4/9 (44%)	NS
Anti-HDV positive	2 (2%)	1/9 (11%)	NS
Anti-HCV positive	11 (13%)	2/9 (22%)	NS

Quantitative variables are expressed as mean (SD). Categorical variables are expressed as n (%).

Table 3 Baseline features of patients with liver cirrhosis and hepatocellular carcinoma according to the presence or absence of HGV-RNA sequences in serum

Characteristics	HGV-RNA negative (n=131)	HGV-RNA positive (n=5)	p Value
Age (y)	64 (9)	57 (9)	NS
Sex (male/female)	88/43	3/2	NS
Aetiology of cirrhosis			NS
Alcoholic	11 (8%)	1 (20%)	
HCV infection	78 (60%)	2 (40%)	
HBV infection	4 (3%)	0	
HCV and HBV infection	5 (4%)	0	
Alcoholic and HCV infection	20 (15%)	1 (20%)	
Alcoholic and HBV infection	2 (2%)	0	
PBC and HCV infection	0	1 (20%)	
Cryptogenic	11 (8%)	0	
Presumed source of infection			NS
Blood transfusion	35 (27%)	4 (80%)	
Unknown	96 (73%)	1 (20%)	
WBC count ($\times 10^9/l$)	5.9 (4.1)	5.4 (2.4)	NS
Platelet count ($\times 10^9/l$)	107 (53)	107 (58)	NS
AST (IU/l)	115 (130)	149 (120)	NS
ALT (IU/l)	106 (117)	158 (121)	NS
Alkaline phosphatase (IU/l)	290 (239)	247 (119)	NS
γ -glutamyl transferase (IU/l)	118 (145)	149 (114)	NS
Bilirubin (mg/dl)	2.2 (2.2)	3.3 (3)	NS

Quantitative variables are expressed as mean (SD). Categorical variables are expressed as n (%). PBC, primary biliary cirrhosis.

cally significant. As shown in table 2 comparison of HGV infected and non-infected patients did not show significant differences concerning demographic features, biochemical and haematological abnormalities, severity of liver lesions, level of detectable HBV replication, or infection with other hepatotropic viruses such as HDV and HCV. The presumed source of infection was also similar. However, a high rate of HGV infection (four of 10) was observed among HBV infected male homosexuals.

HGV INFECTION IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

HGV RNA was detected in two of 104 patients (1.9%). Statistical analysis was not possible due to the small number of HGV-RNA positive patients. One was a 39 year old woman without clinical evidence of liver disease and slightly abnormal liver function tests (serum aspartate aminotransferase (AST) 60 IU/l, ALT 38 IU/l, alkaline phosphatase 149 IU/l, γ -glutamyl transferase 76 IU/l, bilirubin 0.8 mg/dl, serum albumin 42 g/l, prothrombin time 100%) in whom liver biopsy showed mild fatty infiltration of the liver. The other was a 51 year old man with hepatic cirrhosis and abnormal liver function tests. None had a history of blood transfusion or intravenous drug misuse. Eight of 102 HGV-RNA negative patients had received blood transfusions in the past.

HGV INFECTION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

HGV RNA was detected in five of 136 patients (3.7%). This prevalence is almost identical to that found in blood donors. HGV infected patients tended to be younger than non-infected patients. A history of blood transfusion prior to diagnosis of liver disease was more frequent in HGV infected patients. However, statistical analysis did not show significant differences between the two groups of patients concerning demographic features, presumed aetiology of underlying cirrhosis, presumed source of infection, or haematological and biochemical features (table 3). HGV-RNA was detected in association with HCV infection in four of the five cases.

HGV INFECTION IN PATIENTS WITH CRYPTOGENIC CHRONIC LIVER DISEASE

HGV RNA was detected in two of 24 patients (8.3%). One was an asymptomatic 55 year old woman with slight ALT elevation lasting for seven years after recovery from an episode of fulminant hepatitis of unknown aetiology. The patient had received blood products at that time. The other was a 19 year old man with abnormal liver function tests in whom liver biopsy showed evidence of chronic hepatitis with mild activity and minimal fibrosis. A possible source of infection was not identified in this patient. Further analysis was not possible due to the small number of HGV infected patients detected in this group.

Discussion

HGV, or GBV-C, is a recently identified member of the Flaviviridae family that can cause

acute and chronic infection in man.^{1,2} The recent development of sensitive laboratory techniques for determination of HGV RNA sequences in clinical specimens has led to intensive investigation of the frequency and meaning of HGV infection in different clinical conditions. Despite many efforts, however, important aspects of the epidemiology and pathogenicity of HGV infection and its role in acute and chronic liver diseases still remain obscure.

In the current study we investigated the presence of HGV RNA infection in a large series of patients with a variety of chronic liver diseases and in a group of volunteer blood donors living in the same geographical area. The main demographic, epidemiological, clinical, and histopathological features in HGV infected and non-infected patients were analysed.

It is now clear that patients heavily exposed to blood and blood products, such as haemophiliacs, thalassaemics, and liver transplanted patients,^{6,10,13,21,22} as well as those at high risk of parenteral exposure, such as patients on haemodialysis^{23,24} and intravenous drug users,^{3,25} have the highest prevalence of HGV infection. These data suggest that parenteral exposure plays an important role in the transmission of HGV. This study showed, however, that a substantial proportion of HGV infected patients with chronic liver disease did not have a history of overt parenteral exposure, suggesting that HGV could also be transmitted by other routes. Furthermore, irrespective of the clinical or histological diagnosis, most of these patients also had evidence of infection by other parenterally transmitted hepatotropic viruses, such as HCV, HBV, or both, suggesting that HGV may also spread through non-apparent parenteral exposure. Interestingly, four of the nine patients coinfecting with HGV and HBV were male homosexuals and did not have associated risk factors. This observation suggests that HGV may be sexually transmitted, as recently reported.²⁵

Despite a similar epidemiological background, the prevalence of HGV infection in patients with chronic hepatitis C observed in this study was approximately two times higher than that in those with hepatocellular carcinoma, most of whom were also infected with HCV. The reason for this difference is unclear. One possible explanation could be that chronic HGV infection may subside with time, leading to clearance of HGV RNA, as seems to occur in patients with haematological disorders⁶ and in intravenous drug misusers infected with HIV.²⁵ This is likely because the patients with cancer were older and, possibly, had more long lasting viral infection than those with chronic hepatitis. The observation that HGV RNA was detected in young rather than in middle aged blood donors, further supports this hypothesis.

Data on the pathogenic effects of HGV are controversial. HGV infection is the only agent identified in some patients with acute sporadic non-A, non-E hepatitis,² idiopathic fulminant hepatitis,^{7,8} or cryptogenic chronic liver disease.¹⁴ Studies in blood donors² and in

haemodialysis patients,^{4,23} however, have clearly shown that HGV infection is frequently found in subjects without clinical or biochemical evidence of liver disease. In a recent study in haemodialysis patients, HGV infected patients did not usually present with liver abnormalities except if coinfecting with other hepatotropic viruses.²⁴

Mild elevation of ALT is often found in HGV infected blood donors.² In this study, a minimal elevation of ALT was observed in one of the six blood donors in whom HGV RNA was detected in serum, but this abnormality was also observed in a similar proportion of HGV non-infected donors and may be a non-specific finding.

In agreement with previous observations,^{18,26,27} infection with HGV did not appear to increase the severity of liver lesions in patients with chronic liver disease resulting from HBV or HCV infection. On the other hand, there is increasing evidence indicating that coexisting HGV infection does not appear to modify the response to interferon in patients with chronic hepatitis C.^{18,26,27} These data support the hypothesis that HGV coinfection does not play a relevant role in the pathogenesis of HBV or HCV induced chronic liver disease. Further evidence against the pathogenicity of HGV was recently provided by a study of patients with acute post-transfusional hepatitis, showing that combined infection with HGV and HCV did not produce more severe hepatitis than infection with HCV alone.²⁸

Although HGV infection often becomes chronic, chronic hepatitis does not appear to develop in subjects persistently infected with HGV alone, as recently shown in patients with acute non-A, non-E hepatitis.²⁹ In the current study, the prevalence of HGV RNA in patients with chronic liver disease unrelated to HCV or HBV infection, including patients with cryptogenic disease and patients with cryptogenic cirrhosis and hepatocellular carcinoma, was similar to that observed in healthy volunteer blood donors and in patients with alcoholic liver disease. Thus, HGV does not seem to be involved in cryptogenic liver diseases in our geographical area.

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