JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1997, p. 767–768 Vol. 35, No. 3
0095-1137/97/$04.00+0

Prevalence of Hepatitis G Viremia among Healthy Subjects, Individuals with Liver Disease, and Persons at Risk for Parenteral Transmission

HEINZ-HUBERT FEUCHT,[1]* BERNHARD ZÖLLNER,[1] SUSANNE POLYWKA,[1] BÄRBEL KNÖDLER,[2] MATTHIAS SCHRÖTER,[1] HARTMUT NOLTE,[3] AND RAINER LAUFS[1]

Institute of Medical Microbiology and Immunology[1] and Department of Transfusion Medicine,[2] Universitätskrankenhaus Eppendorf, Hamburg, and Department of Internal Medicine, Hospital Pinneberg, Pinneberg,[3] Germany

Received 15 August 1996/Returned for modification 28 October 1996/Accepted 2 December 1996

The prevalence of hepatitis G virus (HGV) and hepatitis C virus (HCV) infection was determined by reverse transcription-PCR in 777 individuals with and without risk factors for viral transmission via blood. From our results we conclude that transmission of HGV and that of HCV are favored by similar risk factors.

Hepatitis A virus, B virus, and C virus (HCV) are the most causative agents of infectious viral hepatitis worldwide. Hepatitis A virus is responsible for 32% of these infections, hepatitis B virus is responsible for 44%, and HCV is responsible for 20% (3). In about 4% of infectious hepatitis cases the causative agent is unknown (2). A new virus, named hepatitis G virus (HGV), has been detected recently by molecular biological methods (9). This virus is closely related to another recently detected virus, named GB virus type C (11). HGV is a member of the family *Flaviviridae*. It is closely related to another virus that causes infectious hepatitis in humans: HCV (8, 10). To determine the prevalence and distribution of HGV, we examined patients with and without risk factors for viral transmission via blood who had biochemical signs of hepatitis. Healthy subjects without risk factors served as a control group. HGV and HCV PCR results were compared.

Sera of 777 individuals from Hamburg, Germany, were tested for the presence of HGV by reverse transcription-PCR. Of these, sera of 154 patients had elevated alanine aminotransferase levels (ALT) (>45 U/liter) and were sent to our laboratory with suspected viral hepatitis. They tested negative for hepatitis A-E virus and human immunodeficiency virus (HIV) infection and had no known risk factors. Three hundred sixty-six patients were at risk for parenteral viral transmission: intravenous drug users (IVDU), recipients of transfusion of blood or blood products, hemodialysis patients, and patients infected with HIV or HCV. In contrast, 257 healthy individuals with normal ALT levels (<30 U/liter) and without risk factors served as a control group.

RNA was isolated from sera by a modified guanidinium thiocyanate-phenol-chloroform method as recently described (5). After reverse transcription with a cDNA primer from the helicase coding region of HGV (H4: 5′-CTCAAGCTTGAGA GCGCATCAGT) (nucleotide positions [np] 4472 to 4460; according to a recently published sequence [9]), amplification was performed in a buffer of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 160 μM (each) deoxynucleoside triphosphate, 30 pmol of both sense (H1: 5′-CACGAATTCTATGG GCATGG) (np 4278 to 4288) and antisense (H4) primers, and 2 U of Amplitaq (Perkin-Elmer Cetus, Emeryville, Calif.): denaturation for 30 s at 94°C, annealing for 60 s at 55°C, and extension for 60 s at 72°C. In a second nested PCR, 30 pmol of inner sense primer (H2: 5′-CTCGAATTCATGCGGACCGG) (np 4305 to 4315) and 30 pmol of inner antisense primer (H3: 5′-CTGAAGCTTCCATCTTTGATGAT) (np 4419 to 4406) were used. Amplification products were separated by electrophoresis in 2% NuSieve 3:1 agarose (FMC, Rockland, Maine) and blotted onto positively charged nylon membranes (Qiagen, Hilden, Germany). After hybridization to a radioactively labeled probe (H8: 5′-TGGCCGGCCAGTTC) (np 4360 to 4375), membranes were exposed to Kodak X-Omat-AR films for 3 h. HCV PCR was performed as recently described using primers of the 5′ untranslated region (6). The methods for RNA extraction and reverse transcription and the conditions for amplification by PCR were the same as described above.

In the group of individuals without risk factors for viral transmission by blood, there was an identical rate of detectable HGV viremia (1.9%) in healthy subjects and in individuals with suspected non-A-E hepatitis (Table 1). In the group of patients with risk factors for viral transmission via blood, we found a high rate of HGV (6.8 to 35.2%) and HCV (10.2 to 35.2%) viremia (Table 1).

Three main results can be derived from our data. First, a high rate of detectable HGV viremia (1.9%) was observed both among healthy individuals and among patients with non-A-E hepatitis. In contrast, the prevalence of HCV viremia as detected by PCR was 0% among individuals without risk factors, despite a reported prevalence of anti-HCV activity of 0.25 to 0.36% by a first-generation assay (1, 4). The small sample size (n = 411) used in this study may be responsible for the absence of detectable HCV RNA among individuals without risk factors (Table 1). Second, identical HGV seroprevalence rates in individuals with and without biochemical evidence of liver disease indicate that HGV may cause infections but may not significantly contribute to viral hepatitis. Third, we found that known risk factors for HCV infection are linked to high prevalence of HGV infection. HGV and HCV viremias were detected concurrently in 3.4 to 24.4% of patients, depending on risk factors (Table 1). In all risk groups about one of four HCV-infected patients revealed detectable HGV viremia.

In our study we found that individuals with hemophilia have the highest risk for HGV infection (Table 1). This group of patients underwent chronic treatment with blood products (hemophilia). Also, IVDU have a high risk for HGV infection which may be due to the practice of needle sharing. Both

* Corresponding author. Mailing address: Institute of Medical Microbiology and Immunology, Universitätskrankenhaus Eppendorf (UKE), Martinistr. 52, 20246 Hamburg, Germany.

TABLE 1. Rates of HGV and HCV viremia in different groups

Group (n = 777)	% Positive (no. positive/total no.) by:		
	HGV PCR (n = 86)	HCV PCR (n = 180)	HGV and HCV PCR (n = 46)
Without risk factors			
Healthy individuals (ALT < 30 U/liter)	1.9 (5/257)	0	0
Individuals with non-A-E hepatitis (ALT > 45 U/liter)	1.9 (3/154)	0	0
With risk factors			
Hemophilia patients	35.2 (6/17)	35.2 (6/17)	11.8 (2/17)
IVDU	28.8 (17/59)	54.2 (32/59)	13.6 (8/59)
Individuals with HCV infection	24.4 (29/119)	100 (119/119)	24.4 (29/119)
Blood transfusion recipients	21.1 (12/57)	14.0 (8/57)	3.5 (2/57)
Individuals with HIV infection	18.2 (10/55)	16.4 (9/55)	5.5 (3/55)
Hemodialysis patients	6.8 (4/59)	10.2 (6/59)	3.4 (2/59)

groups of individuals, those with hemophilia and IVDU, were also found to have the highest risk for HCV infection (Table 1). Therefore, we conclude that besides vertical transmission (7), horizontal transmission via blood is an important route to acquire HGV infection.

REFERENCES

1. **Allain, J. P., P. J. Coghlan, K. G. Kenrick, K. Whitson, A. Keller, G. J. Cooper, D. S. Vallari, S. R. Delaney, and M. C. Kuhns.** 1991. Prediction of hepatitis C virus infectivity in seropositive Australian blood donors by supplemental immunoassays and detection of viral RNA. Blood **78:**2462–2468.
2. **Alter, M., M. Gallagher, T. Morris, L. Moyer, K. Krawczynski, Y. Khudyakov, H. Fields, J. Kim, and H. Margolis.** 1996. Epidemiology of non A-non E hepatitis, p. 34. *In* IX Triennial International Symposium on Viral Hepatitis and Liver Disease, Rome, Italy.
3. **Alter, M. J., H. S. Margolis, K. Krawczynski, F. N. Judson, A. Mares, W. J. Alexander, P. Y. Hu, J. K. Miller, M. A. Gerber, R. E. Sampliner, E. L. Meeks, and M. J. Neach.** 1992. The natural history of community-acquired hepatitis C in the United States. N. Engl. J. Med. **327:**1899–1905.
4. **Dawson, G. J., R. R. Lesniewski, J. L. Stewart, K. M. Boardway, R. A. Gutierrez, L. Pendy, R. G. Johnson, X. Alcade, K. V. Rote, S. G. Devare, W. G. Robey, and D. A. Peterson.** 1991. Detection of antibodies to hepatitis C virus in U.S. blood donors. J. Clin. Microbiol. **29:**551–556.
5. **Feucht, H. H., B. Zöllner, and R. Laufs.** 1995. Comparison of conventional autoradiography with a new DNA enzyme immunoassay for the detection of hepatitis C virus-polymerase chain reaction amplification products. J. Virol. Methods **55:**105–110.
6. **Feucht, H. H., B. Zöllner, S. Polywka, and R. Laufs.** 1995. Study on the reliability of commercially available hepatitis C virus antibody tests. J. Clin. Microbiol. **33:**620–624.
7. **Feucht, H. H., B. Zöllner, S. Polywka, and R. Laufs.** 1996. Vertical transmission of hepatitis G. Lancet **347:**615–616.
8. **Leary, T. P., S. M. Muerhoff, J. N. Simons, T. J. Pilot-Matias, J. C. Erker, M. L. Chalmers, G. G. Schlauder, G. J. Dawson, S. M. Desai, and I. K. Mushawar.** 1996. Sequence and genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis. J. Med. Virol. **48:**60–67.
9. **Linnen, J., J. Wages, Z.-Y. Zhang-Keck, K. E. Fry, K. Z. Krawczynski, H. Alter, E. Koonin, M. Gallagher, M. Alter, S. Hadziyannis, P. Karayiannis, K. Fung, Y. Nakatsuji, J. W. K. Shih, L. Young, M. Piatak, Jr., C. Hoover, J. Fernandez, S. Chen, J. C. Zou, T. Morris, K. C. Hyams, S. Ismay, J. D. Lifson, G. Hess, S. K. H. Foung, H. Thomas, D. Bradley, H. Margolis, and J. P. Kim.** 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science **271:**505–508.
10. **Ohba, K., M. Mizokami, J. Y. Lau, E. Orito, K. Ikeo, and T. Gojobori.** 1996. Evolutionary relationship of hepatitis C, pesti-, flavi-, plantviruses, and newly discovered GB hepatitis agents. FEBS Lett. **378:**232–234.
11. **Simons, J. N., T. P. Leary, G. J. Dawson, T. J. Pilot-Matias, A. S. Muerhoff, G. G. Schlauder, S. M. Desai, and I. K. Mushawar.** 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. **1:**564–569.