Report

GBV-C infection among patients with hepatitis C virus in the Islamic Republic of Iran: a preliminary report

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Introduction

In the past 30 years, five hepatitis viruses, A, B, C, D, and E, have been identified. Hepatitis A and E viruses are transmitted via the faecal–oral route. They can induce acute hepatitis and, very rarely, chronic hepatitis [*1–5*]. Hepatitis B virus (HBV) and hepatitis C virus (HCV), by contrast, are transmitted by blood and blood products [*4,6*]. They cause acute as well as chronic hepatitis, even leading to cirrhosis and/or hepatocellular carcinoma. Hepatitis D virus, or delta agent, is an exception since it is dependent on HBV, and can modify the natural course of HBV. Acute or chronic hepatitis in which neither non-viral causes, nor infection by any of the aforementioned viruses plays a role, is described as non-A–E hepatitis. The search for other viral etiologic agents led to the recovery of the hepatitis F virus from the faeces of a patient with hepatitis. However, as the finding has not been confirmed by other investigators, the virus has been excluded as a causative agent of hepatitis [*7*].

The isolation of the virus HGV, or GBV (after the initials of the surgeon who first isolated the virus), by two groups of investigators working independently has been of great significance [*2,8*]. Both viruses are positive, single-strand RNA viruses, each containing approximately 9400 nucleotides and a genomic structure resembling that of *Flaviviridae*. The structural and nonstructural regions of HGV/GBV are located at the 5' and 3' ends respectively, exactly the same as *Flaviviridae*. Sequence comparisons of HGV and HCV reveal that they have only 26% homology at the amino acid level [*1,6*].

Of the three GB viruses that have been identified, GBV-A, -B, and -C, two (GBV-A and GBV-B) are most likely tamarin (monkey) viruses, while GBV-C can infect humans. Because of the significant nucleotide homology between GBV-C and HGV, the two viruses are often referred to by the single term, GBV-C/HGV [*8,9*]. In this article the term GBV-C is used throughout.

Results of preliminary studies suggest that GBV-C infection can explain a small,

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Received: 12/03/98; accepted: 20/09/98

but quite distinct, portion of post-transfusion non-A–E hepatitis cases. The virus can also be detected in patients with acute or chronic hepatic diseases of unknown etiology. To date, there is no clear evidence to support the view that GBV-C infection affects the natural course of infection with other viral hepatitis agents. Although the mode of transmission of the virus still remains elusive, most investigators agree that blood and blood products play a role [*10–12*]. Our study is a preliminary investigation into GBV-C infection in the Islamic Republic of Iran, based on serum samples collected from HCV-infected patients.

Patients and methods

A group of 21 patients, in the care of internists, gasteroenterologists, urologists and nephrologists at two teaching hospitals (Shariati, Taleghani) and one private hospital (Mehrad), were chosen for the study. Of the group, 4 patients (19.0% of the total) had chronic hepatic disease, 6 (28.6%) had undergone kidney transplant, 3 (14.3%) were on haemodialysis and 8 (38.1%) were random samples of sera from HCV-positive, healthy, volunteer blood donors.

After testing for anti-HCV with a second-generation enzyme-linked immunosorbent assay (ELISA) (Behring Company, Germany), it was shown that all serum samples were HCV-positive. Of the 21 patients, 7 (33%) had a history of interferon (IFN) therapy. Patients were tested for GBV-C RNA in serum by nested primers deduced from the 5' untranslated region [*1*]. RNA was extracted from serum (100 mL), converted to cDNA with an antisense primer, #G75 (5'-CCTATTGGTCAAGAGAGAG-AT 3'), and amplified by polymerase chain reaction (PCR) with sense primer #G58 (5'-CAGGGTTGGTAGGTCGTAAATCC-3') and #G75 for 35 cycles — 94 °C, 30 seconds; 55 °C, 30 seconds; 72 °C, 60 seconds (8 minutes in the last cycle). One-tenth of the amplification product (242 base pairs) was subjected to a second round of PCR with nested primers, sense #G134 [5'-GGT-CAYCYTGCCACTATAGG-3' (Y = T or C)] and antisense #G131 [5'-AA-GAGAGACATTGWAGGGCGACGT-3' (W = T or A)] for 25 cycles with the same amplification conditions, to obtain fragments of 208 base pairs [*1*].

Results

A two-stage PCR assay of anti-HCV positive sera detected HCV RNA in 15 HCV-positive samples (71.4% of the total), and HBV-C RNA in 6 of the samples (28.6% of the 21 serum samples and 40% of the 15 HCV RNA positive samples).

The frequency of HCV and GBV-C positivity in serum samples and the history of treatment with IFN in each group of patients are shown in Table 1. The 4 patients with chronic hepatic disease, the 3 patients on haemodialysis, the 6 kidney transplant recipients, and the 8 blood donors represented 19.0%, 14.3%, 28.6%, and 38.1% of all serum samples respectively — with the highest prevalence of HCV RNA and GBV-C positivity in blood donors (Figure 1).

Discussion

In our study, 21 serum samples primarily identified as HCV-positive by ELISA II were subjected to a two-stage PCR assay, resulting in the detection of HCV RNA in 15 (71.4%) of them. The difference between the number of cases detected by each technique in our study (ELISA II and PCR) might be ascribed to two factors: first, the

Table 1 **HCV RNA, GBV RNA and previous interferon treatment in patients found positive for HCV by ELISA**

Medical history of patients	HCV RNA		GBV RNA		Interferon therapy	
	No.	%	No.	%	No.	%
CHD (n = 4)	4	100.0	1	25.0	4	100.0
Dialysis (n = 3)	2	66.7	0	0.0	2	66.7
Transplantation (n = 6)	1	16.7	1	16.7	1	16.7
Screening (n = 8)	8	100.0	4	50.0	0	0.0

ELISA = enzyme-linked immunosorbent assay *CHD = chronic hepatic disease*

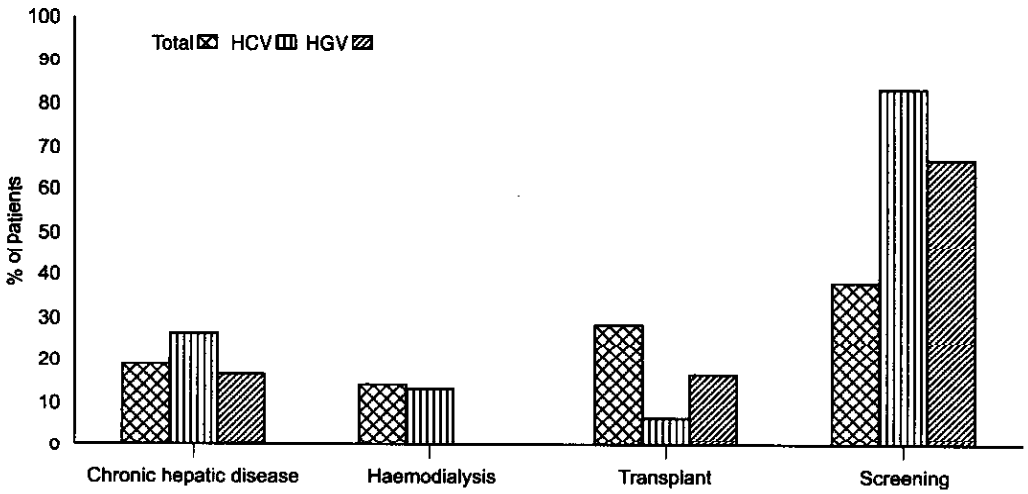

Figure 1 **Rate of infection with HCV and GBV-C among different study groups**

interval between performance of the two tests (mean: 3 years); and secondly, the treatment of some patients with IFN (GBV-C infection has been shown to respond to treatment with IFN).

GBV-C was detected in 28.6% of all samples and in 40% of those that were positive for HCV RNA. After categorizing samples, the prevalence was found to be highest in HCV-positive healthy volunteer blood donors (66.7%), followed by patients with chronic hepatic disease (16.7%) and transplant recipients (16.7%). The sera of patients on maintenance haemodialysis were negative for GBV-C.

Other studies looking at HGV prevalence have reported a relatively lower prevalence than that shown in our study, which detected GBV-C. Tanaka et al. demonstrated HGV and HCV co-infection in 11% of patients with chronic HCV [*13*]. Alter et al. described this co-infection in 20% of pa-

tients with acute viral hepatitis in one study, and in 10% of patients with transfusion-induced hepatitis in another [*7,16*]. Martinol et al. found HGV infection in 21% of patients with chronic HCV and in 32% of injecting drug users [*12*].

Statistics vary regarding the prevalence of GBV-C infection among different groups of patients. In 1996, Masuka et al. found GBV-C infection in 4 of 448 (0.9%) healthy blood donors [*4*]. The prevalence among patients on haemodialysis, as shown by separate studies in France [*2*] and Japan [*3*] was 57.5% and 3.1% respectively. A study in the United States that examined GBV-C infection in kidney transplant recipients showed a 37% prevalence in HCV RNA-negative patients [*6*]. A 1995 study by Hadziyannis et al. reported a prevalence of GBV-C infection in patients with chronic hepatic disease of 21% [*2*]. Aikawa et al. detected GBV-C infection in 24% of injecting drug users with HVC-associated liver disease [*14*].

The difference between GBV-C prevalence in the aforementioned studies and ours may be due to any of three major reasons. First, because of the use of different primers, the methods used for GBV-C RNA identification in various studies might not have been able to distinguish minor differences that exist between the genomic organization of HGV and GBV-C viruses. Secondly, our study looked at the prevalence of GBV-C in HCV-infected patients. This in itself may explain the higher prevalence of GBV-C in these patients compared with normal populations, as it is known that the primary, if not, only mode of transmission of GBV-C is parenterally, through transfusion of blood and blood products. Hence co-transmission of HCV and GBV-C is a probability in our study [*15–19*]. Thirdly, it is clear that the prevalence of GBV-C in study groups can be influenced by the actual prevalence of GBV-C in the population.

In summary, the findings of our preliminary study indicate that the prevalence of GBV-C infection in the Islamic Republic of Iran is similar to that in other parts of the world. Further, the high prevalence of GBV-C among populations with HCV infection probably reflects similar modes of transmission, both intravenously and through exposure to blood and blood products. Future studies should address the role of GBV-C in hepatic diseases and/or in the exacerbation of previous viral hepatic infections. Its actual prevalence in the general population and among high-risk groups should also be determined.

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