



Detection of hepatitis G virus envelope protein E2 antibody in blood donors

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Received 25 September 2006; received in revised form 11 April 2007; accepted 23 April 2007

Corresponding Editor: William Cameron, Ottawa, Canada

KEYWORDS

Hepatitis G virus (HGV);
Blood donor;
Hepatitis B virus
co-infection

Summary

Objectives: The frequency of hepatitis G virus exposure in blood donors varies between 2.5% in Japan to 24.2% in Poland. Therefore there is a geographic difference in distribution of hepatitis G virus (HGV) in the world. We aimed to determine the frequency of HGV exposure in Iranian blood donors.

Methods: Blood samples from 478 Iranian volunteer blood donors were tested. Positive anti-E2 samples were tested for HGV RNA by reverse transcriptase polymerase chain reaction (RT PCR) using primers derived from the NS5A region of the viral genome.

Results: Of the 478 donors enrolled in our study, five (1%) were positive for anti-E2. Only one donor out of a total of three HBsAg-positive donors was co-infected with HGV, but we did not find HGV and HCV co-infection in our subjects. HGV RNA was not observed in the five anti-E2-positive subjects. We did not find HGV viremia and antibody at the same time.

Conclusion: A low frequency of HGV exposure in blood donors was found in this study. We did not observe co-infection of HGV with HCV in our subjects, supporting the theory that although the parenteral route is the most effective means of transmission, other routes such as sexual contact and intra-familial contact may also play a role in HGV transmission.

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Introduction

Hepatitis G virus (HGV) is a single-stranded RNA virus that represents a newly discovered virus belonging to the flavivirus family. Epidemiological data indicate that the virus is

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transmitted via blood/blood products, sexually, and vertically from infected mothers to children.¹ The virus has worldwide distribution with varying prevalence in blood donor populations from different parts of the world, ranging from 1% in the UK² to 18.2% in South Africa.³ Most HGV infections appear to be asymptomatic. The clinical significance of this agent is still uncertain; several studies have failed to demonstrate serious liver disease in patients with HGV viremia,² although the possibility of it having a role in fulminant hepatitis^{4,5} and aplastic anemia has been debated.⁶

In the absence of any reliable serological assay for the diagnosis of infection, HGV RNA detection by reverse transcriptase polymerase chain reaction (RT-PCR) remains the only available method indicating an ongoing HGV infection.⁷

An ELISA has been developed for the detection of antibodies against the HGV envelope protein E2 (anti-E2).⁸ The presence of serum anti-E2 is usually associated with the clearance of serum HGV RNA in patients infected with the virus.^{7,9,10} E2 antibodies are considered useful markers for diagnosing recovery from HGV infections. Anti-E2 can persist for years after clearance of the virus and simultaneous presence of viremia and anti-E2 is very rarely found.⁹ Anti-E2 has been found in >5% of blood donors and in up to 68% of persons in high-risk groups such as intravenous drug users.⁹ In European donors, the prevalence of anti-E2 indicating resolved infection ranges from 3% to 14%.¹¹

HGV has common parenteral risk factors to other hepatitis viruses. The co-infection rate of HGV and HCV in blood donors has been found to be 3.4–24.4% depending on risk factors, and with HBV has been found to be 32%.¹² Co-infection of HGV with other hepatitis viruses may contribute to changes in the evolution of liver disease. But several studies have suggested that HGV co-infection does not seem to affect the course of viral hepatitis.^{9,10,13,14}

To date no country has made a decision to screen HGV in blood donors; however, further studies are necessary to establish the influence of HGV infection on human health and also in the context of other viral infections.

The aim of this study was to determine the frequency of HGV exposure (past infection) in Iranian blood donors as well as co-infection with hepatitis B and hepatitis C virus, and also the co-existence of HGV RNA and anti-HGV.

Patients and methods

This study was performed from April to June 2005 on volunteer blood donors in the city of Tabriz, Iran. Four hundred and seventy-eight blood samples (465 men and 13 women) were collected from donations of unpaid volunteer blood donors; plasma was stored at -80°C and donors interviewed to determine place of residence (455 from urban and 23 from rural areas).

All were tested for liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by routine laboratory techniques. All samples were tested for hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV-Ab), and anti-HGV envelope protein E2 (anti-E2) by enzyme-linked immunosorbent assay (ELISA). The commercial HBsAg enzyme immunoassays kits were the OQPW13 Enzygnost[®] HBsAg 5.0 (Dade Behring, Marburg, Germany),

anti-HCV determination kits were from Avicenna Medical Center (Moscow, Russia), and anti E2 kits were μ PLATE Anti-Hgenv (Boehringer GmbH, Mannheim, Germany). All assay protocols, cut-offs, and result interpretations were carried out according to the manufacturers' instructions. Recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity.

RT-PCR

Anti-E2-positive samples were tested for HGV RNA by RT-PCR using primers derived from the NS5A region of the viral genome. RNA was purified using 200 μl of plasma from each sample using NucleoSpin[®] RNA virus (Machery-Nagel GmbH, Germany) following the manufacturer's instructions. RNA was converted to cDNA using the Surescript One-Step RT-PCR using random hexamer primer at 37°C for one hour. Subsequently, cDNA was amplified, in the same tube, using a denaturation step at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 55°C , and extension at 72°C for 30 s. RT-PCR products were further amplified using nested PCR (94°C for 2 min, followed by 40 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s) using Hot Start Taq polymerase (Roche, Mannheim, Germany). The primers used in the RT-PCR/nested PCR were as follows:

G58 (outer; forward), 5'-CAGGGTTGGTAGGTCGTAATCC-3'

G75 (outer; reverse), 5'-CCTATTGGTCAAGAGAGACAT-3'

G134 (inner; forward), 5'-GGTCAYCTGGTAGCCACTA-TAGG-3'

G131 (inner; reverse), 5'-AAGAGAGACATTGWAGGGC-GACGT-3'

The inner primers should amplify a 208-bp region in the 5'-UTR of HGV (bases 152 through 359).^{15,16} An HGV RNA-positive sample was obtained from Dr Keivan's laboratory (Tehran, Iran) and was used as a positive control. PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and a visualized gel documentation system (Figure 1).

Statistical methods

The laboratory findings and personal data among the groups studied were also compared using Chi-square and t^2 tests using SPSS 11.5 package program. Quantitative variables are expressed as mean $\pm$ standard deviation. A p -value of less than 0.05 was considered statistically significant throughout our data analyses.

Results

From the 478 blood donors enrolled in our study, five (1%) were repeatedly positive for anti-E2. The mean age of blood donors with positive anti-E2 was 33.6 ± 7.3 years and in the negative anti-E2 group was 33.6 ± 9.9 years (not significant). The mean AST levels in the positive and negative anti-E2 groups were 18.2 ± 5.3 IU/l versus 20.9 ± 8.2 IU/l and for ALT, 21 ± 5.1 IU/l versus 24.5 ± 12.9 IU/l, respectively. There was no significant difference between ALT and AST



Figure 1 Agarose gel (1.5%) electrophoresis of PCR products. From right: lane 1, DNA ladder; lane 2, negative control; lane 3, positive control; lanes 4–8, anti-HGV-positive samples.

levels in positive and negative anti-E2 groups. Anti-E2-positive subjects did not show elevation in liver enzymes including AST and ALT levels.

We found nine anti-HCV-positive and three HBsAg-positive blood donors. From the five anti-E2-positive samples, only one was also positive for HBsAg and all were anti-HCV-negative. There was an association between anti-E2 positivity and the presence of HBV marker ($p < 0.031$). Only one donor out of the total of three HBsAg-positive donors was co-infected with HGV, but we did not find co-infection with HCV.

There was no significant difference between the anti-E2-positive and negative samples with regard to age. We did not observe any antibody-positive case in the female donors. All five of the anti-E2-positive donors were from urban areas and there was no positive sample from rural areas (not significant).

HGV RNA was not found in anti-E2-positive cases. We did not find HGV viremia and antibody at the same time.

Discussion

Evidence that HGV is a blood-borne agent warrants studies to determine the prevalence of infection in blood donors in various countries. The first aim of this study was to estimate the rate of exposure to HGV in healthy blood donors as well as those positive for HBV or HCV in Iran. We have shown a low frequency of anti-E2 (1%) in blood donors accepted at the Tabriz blood transfusion center. In European countries, anti-E2 seropositivity in blood donors has been found to range from 10.9% to 24.2%.^{17–20} In South Africa (20.3%)¹⁷ and Brazil (19.5%),¹⁷ even higher anti-E2 prevalence rates have been recorded. In Asian countries,^{17,21} anti-E2 positivity has been found to be significantly lower (2.5–6.3%), although in blood donors in Taiwan, a higher prevalence (10.2%) has been reported.²²

In other countries, anti-E2 has been found in 7.3% of Canadian,²³ 10.5% of Norwegian,²⁴ and 11.3% of Russian blood donors.²⁵ An even higher prevalence of exposure to HGV (52–73%) has been found in several groups at risk of parenteral

exposure to infectious agents, i.e., intravenous drug users, those with a transfusion history, hemophiliacs, hemodialysis patients, and hepatitis C virus (HCV)-positive patients.¹⁹ Most anti-E2-positive patients were found to be HGV RNA-negative and vice versa, indicating an inverse correlation of these two viral markers.¹⁹

The prevalence of HGV RNA in blood donors has been found to range from 1% to 18.2%.^{2,3,26–31} However, Kalkan et al. reported no positivity for HGV RNA in Turkish blood donors.³² Among individuals with parenteral risk factors, the prevalence has been found to be much higher, reaching 18% in hemophiliacs and 16–33% in intravenous drug users.³³

The above studies have shown the wide variations in different geographic areas. These conflicting results on HGV prevalence in blood donors may be related to various factors: the size of the study group, the methods used to describe the HGV, and the demographic and clinical features of the population. Also, differences in host susceptibility or virulence of different strains of HGV may explain the differences.

HGV and HCV infections have frequently been found to be associated and have common parenteral risk factors. The co-infection rate of HGV with HCV in blood donors has been found to be 3.4–24.4% depending on risk factors, and with HBV 32%.¹² In our study, from the three donors positive for HBsAg, only one was also positive for anti-E2, but we did not find HGV and HCV co-infection in our subjects. This is consistent with the report of Praharaj et al.¹² about co-infection of HBV with HGV (29.4%) in blood donors, but contrasts with the results of Tan et al. regarding co-infection of HGV with HCV (33.3%).⁹ Panda et al. also failed to detect any co-infection of HGV with hepatitis B or C.³⁴

Some studies have shown a higher HGV co-infection with HBV and HCV (50%) in blood donors.²⁸ Al-Knawy from Saudi Arabia reported that in patients with HCV infection, the co-infection rate of HGV was 31%,³⁵ higher than that observed in Japan,³⁶ Italy,³¹ and Southern China.²⁹ These findings are in agreement with other collected data on HCV-infected patients^{13,33} and they may reflect a common route of transmission and risk factors of the two agents. Our data regarding HGV and HCV co-infection were not similar to other authors; one possible explanation is the low prevalence of HCV and HGV infection in our subjects and also HGV is capable of independent transmission, indicating that additional routes of viral transmission besides parenteral exposure might exist, possibly sexual, vertical, or even by close contact, as observed in HBV infection. There is a need to systematically investigate the possible risk factors for HGV transmission.

Little information is available to date about the possible influence of dual infection on the course of liver disease. Some authors have discovered that co-infection with HGV has a significant influence on histological findings of HCV hepatitis and that this co-infection could be important for the enhancement of liver damage.^{21,22,37,38} Other studies have shown that in individuals with HGV and HCV co-infection, the HGV infection has no influence on HCV replication or HCV RNA levels, and also it has no influence on the course of liver damage.^{9,14,39–42}

Some authors have found a very high prevalence of HGV infection in chronic hepatitis B patients (CHB).^{43,44} Nevertheless, no evidence has been found that HGV co-infection has any impact on the severity of chronic HBV infection or

changes the course of basic liver disease.^{43–45} However, the significance of HGV infection with other viral hepatitis is still not clear and needs further investigation.

The pathogenicity of HGV seems to be negligible.¹⁴ Our findings agree with previous studies reporting that biochemical evidence of liver inflammation is uncommon in patients with HGV infection.^{14,31,41} None of our HGV-positive subjects had elevated liver enzymes including AST and ALT levels, and we did not expect to observe this because all of our subjects had cleared their infection and did not have viremia.

Most patients infected by HGV show only a minor elevation in aminotransferase levels that lasts until the clearance of HGV RNA.^{14,31} However the clinical implications of HGV infection have not been clearly determined since the vast majority of infected individuals do not show liver injury.

Roth et al. showed a significantly greater prevalence of HGV in urban volunteer blood donors than in rural donors.³⁰ The high prevalence of HGV infection in urban donors suggested specific transmission risks for this group. With respect to parenteral transmission routes of HGV, it is conceivable that urban donors are more likely to have had intravenous injections and to have subjected themselves more frequently to medical (dental procedures, injections, etc.) and non-medical (piercing, etc.) procedures. We did not observe anti-E2 positivity in the blood donors from rural areas in Tabriz. However there were only 23 rural donors (low number) in our study, therefore it cannot be concluded that there was an additional transmission risk for HGV in the urban population of our study.

With regard to the scarcity of information on the prevalence of anti-envelope antibodies coinciding with HGV viremia in Iran, we have used RT-PCR to detect the presence of hepatitis G viremia in anti-E2-positive blood donors. We did not find HGV RNA in our samples and all of our subjects had cleared their infection probably by anti-E2 seroconversion. In a previous study, 4% of drug users and 18% of patients with transfusion-associated hepatitis were found to have both anti-E2 and HGV RNA.⁸ These results are in concordance with the studies published by Tan et al. (in HCV-positive donors) who found that HGV RNA and anti-E2 were mutually exclusive except in 1.5% of donors.⁹

Kondili et al. showed the presence of both markers in one thalassemic patient in Italy.⁴⁶ The temporal overlap between anti-E2 seroconversion and loss of detectable HGV RNA may last more than 6 months.⁴⁶ Anti-E2 has been reported as a marker of recovery from HGV infection, based on observations that HGV RNA and anti-E2 are generally mutually exclusive and that clearance of HGV RNA generally coincides with the appearance of anti-E2 antibody.^{7,9,10} Some studies have shown that only 5% of individuals with any HGV marker are positive for both HGV RNA and anti-E2.¹⁰ More studies are necessary to establish whether the coexistence of HGV RNA and anti-E2 heralds recovery from infection or whether it signals infection with different strains of HGV.

HGV infection seems to occur in all ethnicities all over the world. We observed a low frequency of anti-E2 in Iranian blood donors (1%), and it seems that supervision and screening of blood donors will not be necessary. A more accurate estimation of the prevalence of HGV infection can be achieved with the determination of both HGV RNA and anti-E2. Since only serological tests were performed (and no PCR) in our study, the prevalence rate of HGV has been underestimated and further clinical and epidemiological

studies on the prevalence rate of HGV RNA in blood donors and recipients should be considered.

Acknowledgments

The authors acknowledge the Infectious Disease and Tropical Medicine Research Center of Shaheed Beheshti Medical Sciences University and the Infectious Disease Research Center of Tabriz for financial support of this study.

Conflict of interest: No conflict of interest to declare.

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