Frequency of Hepatitis G Virus Infection among HIV Positive Subjects with Parenteral and Sexual Exposure

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Abstract

**Background.** Epidemiological data indicate that Hepatitis G virus (HGV) is transmitted predominantly through parenteral routes, with a high seroprevalence among injection drug users (IDUs), although sexual transmission has also been reported. In this study our objective was to compare the frequency of HGV infection in two groups of HIV-positive patients including IDUs and those with sexual risk of exposure. **Methods.** Presence of HGV-RNA was analyzed in serum samples from 82 HIV-infected patients including 52 IDUs and 30 cases with sexual (heterosexuals) risk of exposure by reverse transcriptase-nested polymerase chain reaction. Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs), Hepatitis C antibody (anti-HCV), alanine aminotransferase (ALT) levels, HIV viral load and CD4 cells count were also tested in all subjects. **Results.** The overall prevalence of HGV infection was 10.97% in HIV infected patients, with no statistically significant difference between the two groups (13.5% among IDUs vs. 6.7% among the sexual cases). We found a higher frequency of HGV co-infection with HCV in IDUs than in the sexual group (11.5% vs. 3.3%). There was no statistically significant difference between IDUs and the sexual group regarding age, viral load, CD4 cells count, ALT levels and the prevalence of HBV infection. **Conclusion.** The overall prevalence of HGV infection was relatively high in HIV infected patients. HGV-RNA was found more frequently in patients with injection drug use than in those with sexual risk of exposure.

Key words

Human immunodeficiency virus (HIV) – hepatitis G virus (HGV) – injection drug users (IDUs).

Introduction

Hepatitis G virus (HGV) is a single-stranded RNA virus that represents a newly discovered virus belonging to the Flaviviridae family. Epidemiological data indicate that HGV is transmitted predominantly through parenteral routes, with a high seroprevalence among injection drug users (IDUs), although sexual transmission has also been reported [1]. IDUs represent a special subgroup of the population who often share contaminated needles for injection drug use. The rate of blood-borne pathogens infection in IDUs was considerably higher than in the non-IDU population. However, though many people are infected with HGV worldwide, no clear association with a known disease state has been demonstrated [2, 3].

Rami et al found that the overall prevalence of HGV infection was relatively high in both IDU and sexual groups, with no statistically significant difference between them (28% among IDU vs. 32% among the non-IDU) [4].

Although it is well established that HGV is transmitted as a result of exposure to blood, as evidenced by the association of infection with hemodialysis [5], injection drug use [6,7] and vertical transmission [8-10], only partial evidence exists for transmission through sexual activity or exposure to mucosal surfaces [9, 10].

Some studies from Iran [11-13] have reported the prevalence of HGV in hemodialysis patients and blood donors. In a survey by Ramezani et al, 1% of blood donors were exposed to HGV [11].

Due to controversies regarding HGV transmission routes, in this study we aimed to compare the frequency of HGV infection in two groups of HIV-positive patients including IDUs and those with sexual risk of exposure.

Patients and methods

In this cross-sectional study were enrolled 82 HIV positive patients who were referred to the Iranian Research Center for HIV/AIDS in Tehran, Iran. The study subjects were patients with HIV infection who tested positive with two enzyme-linked immunosorbent assay (ELISA) and confirmed with Western blot. Informed consent was obtained
from all subjects.

All patients were tested for Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs) and Hepatitis C antibody (anti-HCV) by ELISA. The used commercial enzyme immunoassay kits were as follows: HBsAg and anti-HBs (Heanostick Biomerieux, Boxtel, The Netherlands), anti-HCV (Biorad, Segrate, Italy). Recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity.

HIV-antibody status was determined by ELISA (MP Biomedicals, Illkirch, France), with positive tests confirmed by the Western blot assay (Diaplus, San Francisco, USA). All assay protocols, cut-offs, and result interpretations were carried out according to the manufacturers’ instructions.

In all patients CD4 lymphocytes counting was performed by flowcytometry and defined as cells/mm3. Serum HIV RNA copy numbers were determined by using real time PCR method (Primer Design Ltd, Millbrook Technology campus, Southampton, UK). The sensitivity limit of the assay was 102-108 copies of target template.

**HGV-RT-PCR**

RNA was extracted from 200 μl of serum using High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer’s instructions. RNA was converted to cDNA using the 1st strand cDNA synthesis kit (Roche Diagnostics GmbH, Mannheim, Germany). Subsequently, cDNA was amplified, using a denaturation step at 94°C for 4 min, followed by 40 cycles of denaturation step at 94°C for 35 sec, annealing for 45 sec at 56°C, and extension at 72°C for 45 sec. RT-PCR products were further amplified using nested PCR (94°C for 4 min, followed by 30 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec) using Taq polymerase (Roche Diagnostics GmbH, Mannheim, Germany). The primers used in the RT-PCR/nested PCR were as follows:

G1 (outer; forward),

5'-AAAGGTGGTGATGGTGATGAC-3',

G2 (outer; reverse),

5'-GCCACCGCCTCACCC-3',

G3 (inner; forward),

5'-TTGGTGGTAGGTCGTAAATCCCG-3',

G4 (inner; reverse),

5'-AGCTGGGGTGCCCATGC-3',

The inner primers should amplify a 333 base pair (bp) region in the 5'-UTR of HGV (bases 134 through 467). PCR products were electrophoresed on a 1.5% agarose gel, containing ethidium bromide and visualized on gel documentation system.

**Statistical analysis**

The Chi-square and t²-tests were used with the SPSS 11.5 Package program for statistical analysis (Chicago, IL., USA). Data are presented as mean ± SD or, when indicated, as an absolute number and percentage. A p-value of < 0.05 was considered significant.

**Results**

A total of 82 HIV positive patients with the mean age 37.4±8.6 (range: 23-60) years were enrolled in the study: 68.3% of patients were male and 31.7% were female. The mean CD4 cells count was 347.15±175.97 cells/mm³. The mean log₁₀ HIV viral load was 1.98±2.05. The overall prevalence of HGV infection was 10.97% in HIV infected patients. 88.9% of patients with HGV had a CD4+ count ≥200 cells/mm3 and 11.1% had a CD4+ count <200 cells/mm³.

HIV-positive patients were divided in two groups according to their main risk factor for HIV-1 acquisition including IDUs and those with sexual risk of exposure. None of the IDU patients were sexually exposed.

IDUs group consisted of 51 males and 1 female with mean age 37.7±8.4 years. The mean CD4 cells count was 340.4±202.9 cells/mm³. The mean log₁₀ HIV viral load was 2.09±2.07. HGV-RNA was positive in 13.5% of patients.

Those with sexual risk of exposure included 5 males and 25 females with mean age 36.9±9.1 years. The mean CD4 cells count was 358.6±119.1 cells/mm³. The mean log₁₀ HIV viral load was 1.77±2.04. HGV-RNA was positive in 6.7% of patients.

There was no statistically significant difference between IDUs and the sexual group regarding age, viral load, CD4 cells count, ALT levels, and the prevalence of HGV, HBV, and HCV infection. We found a significant difference between the IDUs and the sexual group regarding sex and anti-HCV (p<0.001).

Demographic and clinical characteristics of HIV positive subjects with parenteral and sexual exposure were shown in Table I.

**Discussion**

In this study the frequency of HGV infection was determined in two groups of HIV-positive patients including IDUs and those with sexual risk of exposure. HGV RNA was found more frequently in patients with injection drug use than in those with sexual risk of exposure.

Most of the patients with HGV had a CD4 count ≥200 cells/mm³ and only 11.1% had a CD4 count <200 cells/mm³. However, due to the limited number of the patients in the
current study, a conclusion can not be reached regarding the association of HGV viremia and prolonged survival among HIV patients.

The predominant route of transmission of HGV appears to be parenteral by contaminated blood and blood products and intravenous drug use, although other routes such as sexual and vertical transmission or through saliva may also exist [14-17].

Rami et al found that the overall prevalence of HGV infection was relatively high in both IDU and sexual groups, with no statistically significant difference between them [4]. In a study by Campo, HGV-RNA was positive in 12 out of the 37 HIV infected patients (32.4%). He reported that the prevalence of HGV infection was high in the HIV infected IDUs [18]. In another investigation by Frey et al, in a comparison of the subjects with sexually transmitted disease (STD) versus those without STD, the prevalence of HGV was 11.3% versus 4.9%, on the basis of PCR results alone, and 36.6% versus 8.8%, when results of PCR and enzyme linked immunosorbent assay were combined. Sexual activity and, possibly, the presence of STD increased the risk of HGV transmission [1].

Bourlet et al studied 80 subjects infected with HIV, divided into four groups of 20 individuals each, according to their main risk factor for HIV-1 infection: blood product recipients (group 1), intravenous drug users (group 2), homosexuals (group 3), or heterosexual exposure (group 4). The overall prevalence of HGV infection was 66.3%. No significant difference was observed in HGV prevalence among the four groups (75, 75, 55, and 60%) in groups 1, 2, 3 and 4, respectively) [19].

The other surveys reported a relatively high prevalence of HGV-RNA among non—injecting drug—using homosexual and bisexual men and heterosexual persons [20, 21]. The risk of acquiring HGV infection increased with prolonged duration of work as a prostitute [22].

Zehender et al showed a considerable spread of HGV among Italian HIV-positive IDUs and indicated that HGV infection does not enhance liver impairment [23].

In our study, the overall prevalence of HGV infection was relatively high in HIV infected patients. Patients with HIV and HCV co-infection were more likely to be HGV-RNA positive. HGV-RNA was found more frequently in patients with injection drug use than in those with sexual risk of exposure. Our findings were in agreement with Campo et al [18] and Zehender et al [23] studies but in contrast with Frey et al [1] reports. These conflicting results may be related to various factors such as the size of the study group and social features and lifestyle of the subjects. Further studies are needed to explain the modes of HGV acquisition in HIV infected patients.

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Conflicts of interest

None to declare.

References


