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# Hepatitis G virus and its prevalence and genotypes in patients with hepatitis B and C in Ahvaz, southwestern Iran

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**Aim:** The aim of this study was to determine the prevalence and genotype distribution of hepatitis G virus (HGV, or GB virus C) in patients with hepatitis B and C in Ahvaz, southeastern Iran.

**Materials and methods:** A total of 100 patients were selected; 50 were positive for hepatitis C virus (HCV) and 50 for hepatitis B virus (HBV). HGV status was examined among the HCV- and HBV-positive patients. An enzyme-linked immunosorbent assay (ELISA) kit was used to detect anti-E2 antibody. Nested reverse transcription polymerase chain reaction (RT-PCR) was used for RNA detection from serum samples of chronic hepatitis patients. Sequencing was done to determine genotypes

**Results:** Using the ELISA method, co-infection of HGV with HCV and HBV was determined at 6% and 8%, respectively. RT-PCR showed that co-infection of HGV with HCV and HBV was 22% and 10%, respectively. Based on nucleotide sequencing of PCR products, the predominant genotype of HGV among the samples was 2a.

**Conclusion:** Our study showed that the co-infection rate of HGV in patients with hepatitis B and C was somewhat high and was higher in HCV- than in HBV-infected patients. As our findings and other reports from Iran and neighboring countries indicate, genotype 2 of HGV may be the most common genotype of HGV in Middle Eastern countries.

**Key words:** Hepatitis G virus, hepatitis B virus, hepatitis C virus, prevalence, genotype, reverse transcription polymerase chain reaction

## 1. Introduction

Hepatitis G virus (HGV, or GB virus C) is an enveloped positive-sense, single-stranded virus belonging to the family *Flaviviridae* (1). This virus is transmitted via blood and blood products, like the hepatitis C virus (HCV) (2). HGV has a worldwide distribution among those with multiple sexual partners and drug abusers (3). Based on analysis of the 5' noncoding region (NCR) or E2 sequence, there are 5 major genotypes and a recently identified sixth genotype, all distributed distinctly in different geographical regions (4). Transfusion-related infections are of the life-threatening kind. Hepatitis was the first disease related to transfusion and was first identified by Beeson in 1943 (5). Although HGV belongs to *Flaviviridae*, unlike HCV, it seems that it is not involved in hepatitis (6). The rate of hepatitis B virus (HBV) infection is very high with approximately 350 million carriers in the world, constituting a serious public health problem (7). HCV infection is the most important cause of chronic liver disease with a total of over 200 million cases of HCV worldwide (8). Co-infection of HGV with HCV is very common, and to a lesser degree with HBV (9). Several studies have shown that its co-infection with human immunodeficiency virus (HIV) is associated with a reduced mortality rate and enhanced reduction of HIV viral load in response to the highly active antiretroviral therapy regime (10,11).

HGV infection could remit because of the disappearance of RNA at the time of antibody production. Most patients that have anti-E2 antibody are negative for HGV RNA, which suggests an inverse correlation of these 2 viral markers (12,13). Anti-E2 antibody can be distinguished in the beginning of infection and also later, along with HGV RNA, suggesting a window period between clearance of RNA and reappearance of anti-E2 antibody (14). There is an enzyme-linked immunosorbent assay (ELISA) test for the

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detection of anti-E2 antibody and a reverse transcription polymerase chain reaction (RT-PCR) test for the detection of RNA to diagnose HGV infection (15,16). The aim of this study was to epidemiologically survey prevalence and genotype distribution of HGV in patients with hepatitis B and C in the southwest of Iran. The presence of anti-E2 antibody and RNA in sera was tested, and sequencing was performed to distinguish genotypes of HGV in HBV- and HCV-positive patients.

## 2. Materials and methods

### 2.1. Study population

This study was conducted between July 2011 and December 2012. A total of 100 patients were included in the study. First, the patients with chronic hepatitis B and C were detected, and then their sera were collected from different diagnostic laboratories. Altogether 100 samples were collected, 50 HCV- and 50 HBV-positive. Serum samples of each patient were aliquoted into 2 microtubes. One tube was stored at –20 °C for the ELISA test and the other at –80 °C for RT-PCR.

### 2.2. Serological assays

HCV-Ab and HBs-Ag were detected with ELISA kits (Dia.Pro, Italy) according to manufacturer instructions. Anti-E2 antibody was detected with an IgG ELISA kit (Cusabio, Japan).

### 2.3. RNA extraction and cDNA synthesis

RNA was extracted from 200 µL of each serum using the High Pure Nucleic Acid Kit (Roche, Germany). The extracted RNA was used to synthesize complementary DNA by cDNA preparation kit (Fermentas, Lithuania); the prepared cDNA was stored at –20 °C.

### 2.4. PCR amplification of HGV RNA

Nested PCR using 4 primers designed from the 5' untranslated region (5'-UTR) of HGV (Table 1) was used for HGV RNA amplification (17). In the first amplification step, 5 µL of cDNA sample was amplified in a 25-µL reaction volume containing 2.5 µL of buffer for PCR reaction (Roche), 0.5 µL dNTP (10 mM), 2.5 µL of $MgCl_2$, 0.15 Taq DNA polymerase (5unite, Roche), and 0.3 µL of 2 outer primers located at position 102 for sense and 457 for antisense; sterile water was used as the rest of the volume. The amounts of ingredients in the next round were similar.

### 2.5. Polymerase chain reaction

Thermal conditions of 35 cycles are shown in Table 2. The thermal conditions for the next amplification step were similar to those of the first, containing 5 µL of the first-round PCR product in 25-µL volumes for PCR reaction. For inner primers, the sense was located at position 134 and antisense at 376. The second-round PCR amplicon containing 261 nucleotides was round in 2% agarose gel, considered as positive for HGV RNA (Figure 1). In each round of nested PCR, RNase DNase-free water and control positive (obtained from Keivan Virology Laboratory, Iran) were used, as well as negative serum as the negative control.

### 2.6. Phylogenetic analysis

Sequencing of nested-PCR products was done by Bioneer Company, South Korea. Genotypes of all products were determined by BLAST with online sequences. The phylogenetic tree was constructed in MEGA 5 software (Biodesign Institute, USA) by the boot-strapping method.

## 3. Results

Table 3 shows the results of the serologic and molecular methods of HGV detection in this project. The mean age of patients with HBV was 35.24 ± 13.89 years with 48% male and 52% female, and 30.28 ± 12.03 years for HCV with 68% male and 32% female. These samples were first tested to determine presence of the anti-E2 antibody, with 7 out of 100 positive. Four of 50 patients with hepatitis B (8%) and 3 of 50 patients with hepatitis C (6%) had antibodies against the HGV E-2 envelope protein.

All samples, including those anti-E2 antibody-positive and -negative, were subjected to PCR testing. HGV RNA

**Table 1.** Four primers of 5'-UTR used in HGV PCR reaction and genotyping.

| | | | |
|---|---|---|---|
| Outer | 102-121 | Sense | 5'-GCCAAAAGGTGGTGGATGGG-3' |
| | 457-477 | Antisense | 5'-CGGAGCTGGGTGGCCCCATGC-3' |
| Inner | 134-153 | Sense | 5'-TGGTAGGTCGTAAATCCCGG-3' |
| | 376-395 | Antisense | 5'-TGGTCCTTGTCAACTCGCCG-3' |

**Table 2.** Temperature and time of PCR steps.

| Step | Preheating | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 45 | 72 | 72 |
| Time (min) | 5 | 1 | 1 | 2 | 10 |

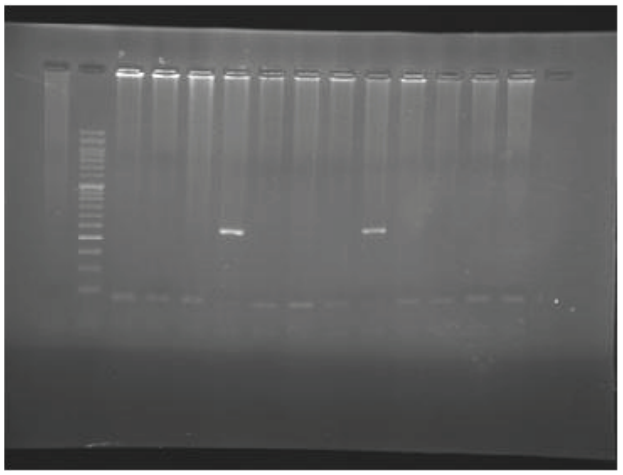

**Figure 1.** The image of HGV RNA PCR product on agarose gel painted with safe staining, observed on a Gel Documenter; the first lane is the negative control, lane 2 is a 50-bp DNA ladder, and lanes 4 and 8 are 2 positive samples with 260-bp nucleic acids, while the others are also negative.

was not found in the negative control, but it was found in 16 out of 100 (16%) patients. In the patients with HBV, 5 out of 50 (10%) had HGV RNA, and in the case of HCV, 11 patients (22%) were positive for RNA. No one had HGV RNA and anti-E2 antibody simultaneously. The mean age of patients co-infected with HGV was 34.21 years. However, there was no significant difference between sexes ($P > 0.05$).

The results of sequencing of 5'-UTR showed that all positive samples were genotype 2a, both co-infected with HCV and with HBV. The phylogenetic tree was constructed with the boot-strapping method using MEGA 5 software, showing 6 genotypes of HGV, including 2a, the most prevalent genotype in Iran (Figure 2).

## 4. Discussion

HGV was detected by RT-PCR in 16% of all cases. ELISA testing showed that 8% of HBV-infected patients and 6% of HCV-infected patients were positive for anti-E2 antibody. The values related to co-infection of HGV with HCV and HBV have been reported by other researchers in Iran as varying between 5% and 43% (9,18,19). Nevertheless, co-infection of HGV has been studied only with HCV, not HBV, in Iran. Ghanbari et al. reported that its prevalence in HCV-infected patients was 43.6% (18), much higher than our findings. Amini et al. reported that 25% of HGV-infected patients also had HCV (9), a value that is close to our findings. Zali et al. reported 40% co-infection of HGV with HCV (19), higher than our findings. This result is consistent with the data reported earlier, but greater than the result of our report (22%). The present study represents the first investigation of HGV infection in patients with chronic hepatitis B living in Ahvaz, Iran. Yang et al. in Taiwan showed that co-infection of HGV with HBV and HCV was 18% and 55%, respectively (14), suggesting that HGV and HCV may share the same route of transmission, in contrast to HBV.

In some studies, co-infection of HGV with HCV and HBV was reported with lower values. Alvarado-Mora et al. in Colombia reported that 5.06% of HBsAg-positive samples were also HGV-positive, while 3.2% of HCV-positive cases were HGV-positive (4), which in the case of HBV samples is close to our findings but much lower than the co-infection rate of HGV and HCV in the present study. Co-infection of HGV with HCV was reported as 12.2% by Hofer et al. (20). In an investigation by Ziaee et al. on hemodialysis patients in Birjand, Iran, 5% of HGV-infected patients also had HCV (21), much lower than our results. Co-infection of this virus with HBV and HCV has been studied by Abo Odeh et al. in the United Arab Emirates, and the figures were 5.7% and 14.3%, respectively (22), close to our findings. Co-infection of HGV with HCV and HBV in Turkey was 7% and 29%, respectively (23), which is dissimilar from the results of the present study. There is a large variation and difference in the prevalence of HGV infection in different geographical regions. This difference may be due to the volume of the population involved in the study, methodology used to detect HGV infection, demographic and clinical features of patients, and different patterns of transmission of virus around the world (blood and blood components, sexual routes, intravenous injection, etc.)

Based on the nucleotide sequence of residue 134-395 of the 5'-UTR region of HGV samples, the genotype 2a was the only genotype of HGV in our city. Our findings are consistent with other studies reported from other

**Table 3.** Demographic data, hepatitis markers, and percentage of HGV RNA and anti-E2 antibody in patients with hepatitis B and C.

| Virus | Patients | Male/female (%) | Mean age (years), male/female | Anti-E2 antibody | HGV RNA |
|---|---|---|---|---|---|
| HBV | 50 | 48/52 | 34.12/36.73 | 4 (8%) | 5 (10%) |
| HCV | 50 | 68/32 | 32.5 / 28.06 | 3 (6%) | 11 (22%) |
| Total | 100 | 100 | 33.31 / 32.3 | 7 (7%) | 16 (16%) |

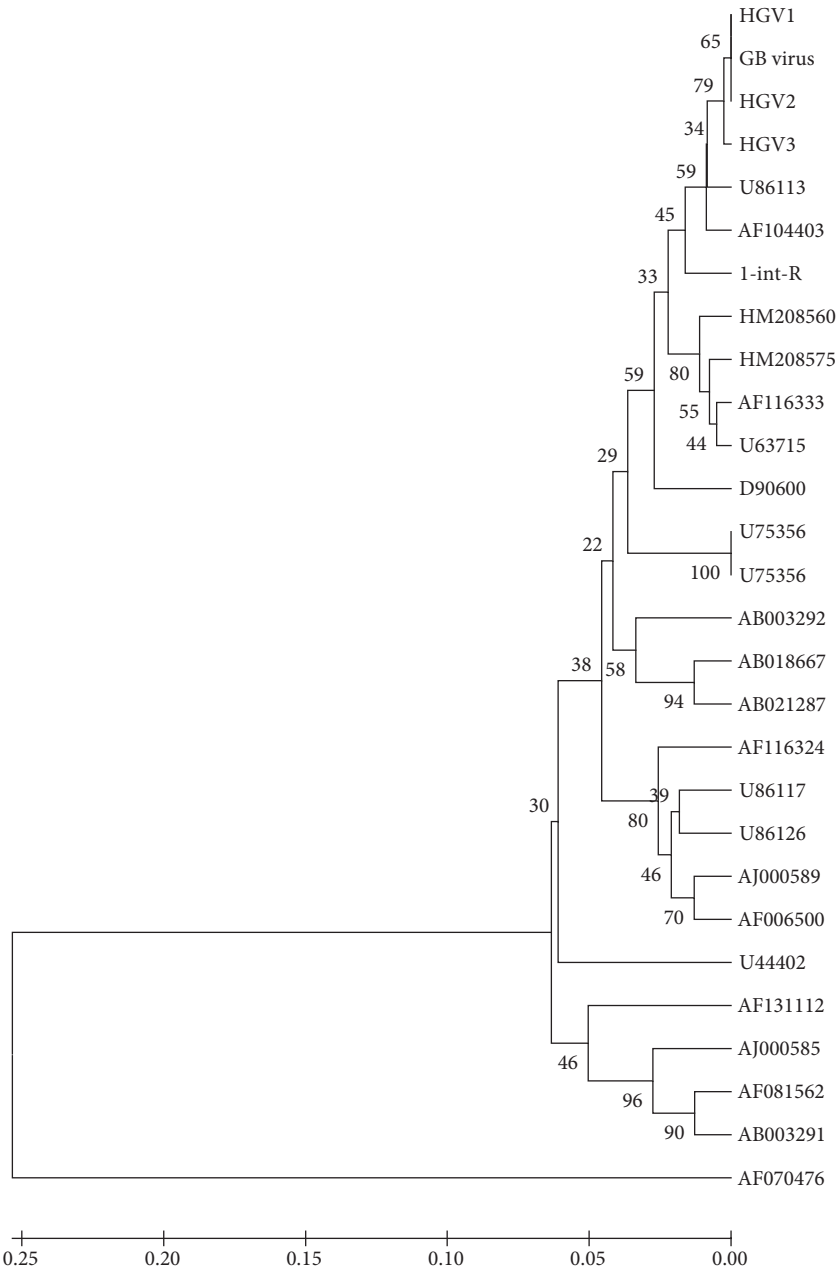


**Figure 2.** Phylogenetic tree of HGV genome constructed by boot-strapping method. The GenBank accession numbers are given.

cities of Iran, as they have reported only genotype 2a (18). Reports from other Middle Eastern countries (United Arab Emirates, Turkey, and Saudi Arabia) showed that genotype 2 was the most prevalent genotype in these countries (22,24,25). Although the aim of this research was to identify the prevalence and genotype of HGV (or GB virus C) in patients with hepatitis B and C, to our best knowledge, this report is the first study to survey co-infection of HGV with HBV in the Iran. We found that the co-infection rate of HGV with HBV in Ahvaz was 10% by RT-PCR and 8% by ELISA, and its genotype was 2a. Our findings show that co-infection of HGV with HCV is much higher than with HBV, and this result is consistent with other studies reported from other countries. High co-infection of HGV with HCV awaits more investigations, however.

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