

## **Prevalence of Hepatitis G virus (HGV) in High-Risk Groups and Blood Donors in Tehran, Iran**

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### **Abstract**

The hepatitis G virus (HGV) is a newly discovered RNA virus, which is associated with acute or chronic hepatitis. A survey was conducted in thalassemic patients, intravenous drug users (IVDU) and blood donors aiming to investigate the prevalence of hepatitis G virus (HGV) infection in these groups in Tehran. The presence of HGV RNA in these populations was determined using reverse transcriptase polymerase chain reaction (RT-PCR) of the 5' non-coding region (NCR) of the virus. One percent of blood donors, 12.9% of thalassemics and 8.8% of the IVDUs, were infected with HGV. Twenty-five percent of HGV positive cases were also positive for HCV and none were positive for HBV-DNA. The greatest proportion of HCV positive cases were seen in IVDU group (67.4%) being the only HIV positive group (8.8%). In conclusion our study showed that HGV infection occurs with relatively intermediate frequency among thalassemics and IVDUs in Iran and a higher rate of HGV-RNA was observed in older IVDUs.

**Keywords:** *GB virus C, Blood donor, IVDU, Thalassemia, HGV, Iran*

### **Introduction**

The hepatitis G virus (HGV) and GB virus (GBV-C) have recently been identified during the search for a possible etiological agent for non-A to E hepatitis (1). The clinical significance of GBV-C infection and its pathogenic role in hepatitis or any other diseases remain unclear. (2)

The HGV consists of a single RNA strand of positive polarity which is distantly related to the hepatitis C virus (HCV) and is associated with acute or chronic infection, predominantly in patients with chronic hepatitis C (1, 3). The sequence comparison showed that GBV-C and HGV had approximately 86% nucleotide sequence and up to 95% amino acid similarities. They both belong to the Flaviviridae family, which also includes the hepatitis C virus. (4, 5) Transmission of the virus by blood or blood products, or other parenteral routes of exposure

such as intravenous drug, has been clearly established.

Studies suggest that HGV infection can explain post-transfusion non-A to E fulminant hepatitis (6) or chronic hepatitis (7). It may be assumed from these data that many HCV positive patients suffer from a parallel GBV-C/HGV infection, presumably occurring due to identical risk factors (8). HGV RNA has also been detected in recipients of blood transfusions (1) or hemodialysis (9) and in intravenous drug users (IVDU) (10, 11). The virus can also be detected in patients with acute or chronic hepatitis infections of unknown etiology (6). So far, there is no clear evidence to support the view that HGV infection affects the natural course of infection with other viral hepatitis agents. But, most investigators agree that blood and blood products play a role in the mode of transmission of the virus (9). A long-term follow-up of pa-

tients with post-transfusion hepatitis has revealed that HGV may be cleared by the patient's immune response (12).

To date, there is no data on prevalence of HGV in blood donors and patients in Iran. The purpose of our study was to evaluate the prevalence of HGV infection in thalassemics and IVDUs as high-risk groups as well as blood donors as control and also to estimate co-infection with HBV, HCV and HIV infection.

## **Materials and Methods**

This study was performed on 165 subjects of high-risk groups and blood donors. The first high-risk group consisted of 31 randomly selected (mean age of 20.8 yr, SD± 8.5) patients with major thalassemia who attended Tehran thalassemia Clinic.

The second high-risk group was 34 intravenous drug users (mean age 29.17yr), who were selected from the Rehabilitation Center in Tehran. The third group was 100 blood donors (mean age 33.29 yr, SD± 15), who were randomly selected from donors attending Iranian Blood Transfusion Services (IBTS) in Tehran. All serum samples were stored at -20° C till use.

### **Serological tests**

All 165 serum samples were tested for hepatitis B surface antigen (HBsAg) using Hapanostika (Organon Teknika), antibody to hepatitis C (anti-HCV) using UBI, HCV EIA4 (Organon Teknika), and HIV antibody using Vironostika kits (Organon Teknika) according to manufacturer's instructions.

Positive sera for HBsAg and HCV by ELISA were also tested for HBV-DNA and HCV-RNA, using PCR. All sera were also tested for presence of hepatitis G virus RNA (GBV-C/HGV) by RT-PCR.

### **PCR detection of HBV-DNA**

Samples were amplified using S region primers (Table 1) as previously described (13).

Visualization of the PCR products was made by ethidium bromide (EtBr) staining in 1.5% agarose gels following electrophoresis. Samples

containing 310 bp PCR products were considered as HBV DNA positive.

### **RNA extraction and cDNA synthesis**

Ribonucleic acids (RNA) were extracted from 250 µl of each serum with Trizol LS reagent (Life Technologies, Gibco-BRL). Complementary DNA synthesis was performed according to the manufacture's instructions (Superscript™ Pre amplification system Life Technologies Gibco-BRL).

### **PCR amplification of HCV RNA**

Samples were amplified in two steps PCR using the 5' untranslated region (UTR) primers (Table1). Samples containing 235 bp PCR products, in agarose gel were considered as HCV RNA positive (14).

### **PCR amplification of GBV-C/HGV-RNA**

Nested PCR using four primers (Table 1) designed from the 5' untranslated region (UTR) of GBV-C was used for HGV RNA (15). In the first amplification step, 5 µl cDNA sample was amplified in a 25 µl reaction volume containing 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris HCl (pH= 8), 200 µM dNTPs, 1 U Taq DNA polymerase (Cinnagen, Iran) and 30 pM of each outer primer located at position 108, and anti sense primer located at 531.

### **Polymerase chain reaction**

Thirty cycles at 94° C for 60 s, 55° C for 70 s and at 72° C for 1.5 min. The condition for the second amplification step was similar to the first one, 20 µl PCR reaction containing 1 µl of first step PCR product. For primers the inner was sense located at position 134, and anti sense primer located at position 467 were used. Samples containing 333 bp PCR products in agarose gel were considered as HGV RNA positive (Fig. 1).

In each step of PCR, sterile water, normal serum control and a negative serum (non reactive on multiple assays) were used as negative controls, and a positive UTR region of HGV cloned plasmid (kindly provided by Dr M N Al-Ahdal) was used as positive control.

## Results

The rate of hepatitis markers (HBsAg, anti-HCV, HGV-RNA) and anti-HIV in thalassemics and IVDUs is shown in Table 2.

As table 2 shows thalassemics and IVDUs had a significant higher HGV-RNA positivity than blood donors (thalassemics  $X^2_{MH}= 9.06$ ,  $P= 0.002$ ; IVDUs  $X^2_{MH}=5.32$ ,  $P= 0.02$ ).

Gender and sex distribution in HGV-RNA positive patients are shown in Table 3. The rate

of HGV-RNA in female thalassemic patients was higher than males, but this rate was higher in males than females in IVDUs group (9.4% vs 0%), although neither was statistically significant.

The highest rate of HGV-RNA was shown in the age group of 21 years and higher (Table 3). Co-infection of HGV either with HCV or with HIV is depicted in Table 4.

**Table 1:** Primer sequences for PCR and RT-PCR

Virus	Gene	Primers name	Type	Sequence	Base position
HBV	Surface	B1	Sence	5' att cct agg acc cgc teg tgt tac	175-199
		B2	Anti sence	5' aat tag agg aca aac ggg caa cat acc	485-458
HCV	5'UTR	C1	Sence	5' ccc tgt gag gaa ctw† ctg tct tea cgc	(-299)-(-272)
		C2	Anti sence	5' ggt gca egg tct acg aga cct c	(-1) - (-23)
		C3	Sence	5' tct agc cat ggc gtt agt r‡y§g agt gt	(-264)- (-238)
		C4	Anti sence	5' cac tcg caa gca ccc tat cag gca gt	(-29) - (- 55)
HGV	5'UTR	G1	Sence	5' agg tgg atg ggt gat	108- 123
		G2	Anti sence	5' tgc cac ceg ccc tea ccc gaa	531- 510
		G3	Sence	5' tgg tag gtc gta aat ccc ggt	134 – 155
		G4	Anti sence	5' ggr gct ggg tgg ccy cat gcw† t	467 – 445

† a or t , ‡ a or g , § c or t

**Table 2:** Hepatitis and HIV markers in thalassemics, intravenous drug users and blood donors

Marker	thalassemics	Intravenous drug users	Blood donors
HBs-Ag	0.0%(0/31)	5.9%(2/34)	0%(0/100)
Anti-HCV	23.3%(7/31)	67.4%(22/34)	0%(0/100)
HCV-RNA	19.35%(6/31)	20.58%(7/34)	0%(0/100)
Anti-HIV	0.0%(0/31)	8.8%(3/34)	0%(0/100)
HGV RNA	12.9%(4/31)	8.8%(3/34)	1%(1/100)

**Table 3:** Relationship between HGV-RNA, sex and age in thalassemics and intravenous drug users

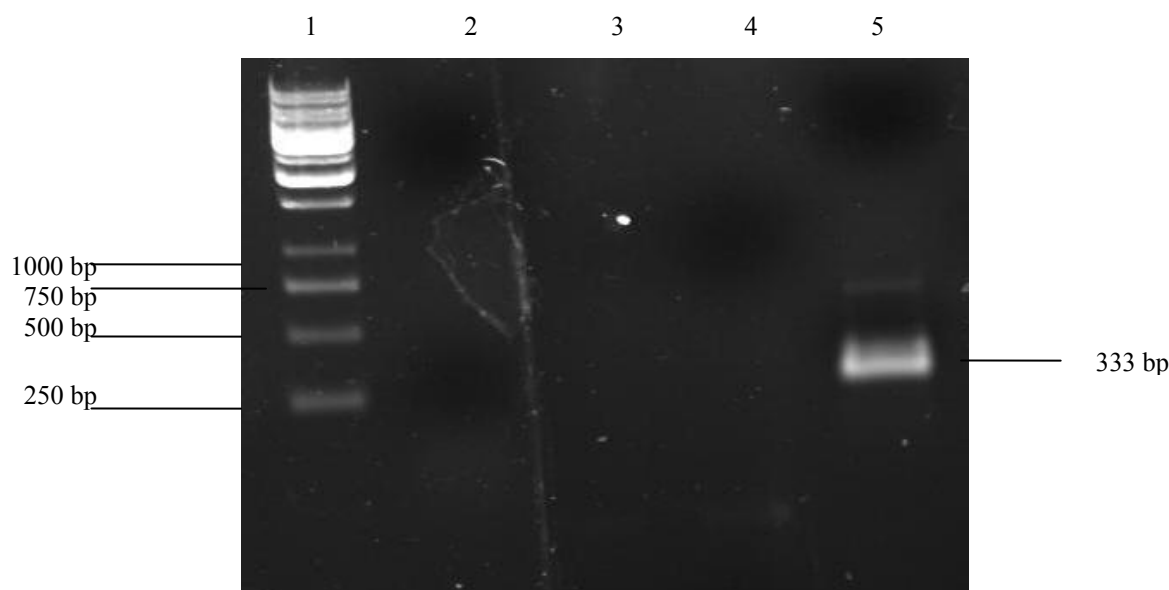
		thalassemics	Intravenous drug users	Fisher test
Sex*	Male	10%(2/20)	9.4%(3/32)	0.645
	Female	18.2%(2/11)	0.0%(0/2)	0.705
Age**	<20	5.26%(1/19)	0.0%(0/3)	0.863
	≥21	25.0%(3/12)	9.67 %(3/31)	0.204

\* Fisher exact test =0.447 for thalassemics and 0.828 for IVDUs

\*\*Fisher exact test= 0.148 for thalassemics and 0.751 for IVDUs

**Table 4:** Co-infection of HCV/HIV in HGV-positive thalassemics and intravenous drug users

Group	HGV/ HCV positive	HGV /HIV positive
thalasseemic	25%(1/4)	0.0%(0/4)
IVDU	0.0%(0/3)	66.6%(2/3)
Fisher exact test	0.57	0.14



**Fig. 1:** PCR amplification of HGV- RNA. Lane1: MW Marker (1kb). Lane2, 3: Negative control serum. Lane 4: PCR control. Lane 5: HGV-RNA positive.

## Discussion

The hepatitis G virus (HGV) is a newly discovered RNA virus, which is possibly transmitted parenterally (1). It is associated with acute or chronic hepatitis and may lead to cirrhosis and liver cancer, characteristics shared by the hepatitis C virus. Hepatitis C virus is prevalent in drug users and thalassemics, but frequency and role of hepatitis G virus in pathogenesis of hepatitis is not yet well established (16).

In Mediterranean Basin, thalassemia is a major cause of multiple blood transfusions (17). An average of 160000 blood units are being transfused to 20000 thalassemic patients per year (18), harbouring a high risk of transmission of blood borne agents such as viral hepatitis and HIV.

In a preliminary report from Iran (19) a rate of 28.6% of GBV-C among a group of patients (chronic hepatitis, kidney transplant, haemodialysis, HCV positive patients and also volunteer blood donors) and 40% co-infection of GBV-C was also reported among patients with HCV.

One percent of the examined blood donors were HGV RNA positive. This result is consistent with the data reported earlier (17, 20) con-

cerning healthy blood donors from Italy and in contrast with other studies (21, 22) who found 7.1% and 9.7% of blood donors were HGV positive in central Brazil, respectively.

HGV RNA was detected in 12.9% of thalassemics, which is similar to 11% reported by Cacopardo et al (17). Moreover, a higher prevalence (19%) of serum HGV-RNA positive has been reported in Greek thalassemic patients (23). This may be explained by a greater number of transfused blood units per year in the latter group since the population examined was made up of patients with thalassemia major only.

It has been suggested that HCV and GBV-C/HGV are transmitted together (24). One remarkable finding in our study was the co-infection of HGV and HCV infection in the thalassemic group (25%).

The HGV-RNA infection rate was less than reported earlier (16, 25, 26) in IVDUs.

We found that HGV infection was more frequent among individuals who were older, than younger group. This indicates a higher risk of infection by increasing age because of more transfusing of blood and blood products and

increasing the chance of infection with blood borne viruses. Contrary to our findings, Shev et al found no age relation in GBV-C/HGV positive and negative patients in a group of 22-53 yr old patients with chronic hepatitis (27).

Our finding regarding HBsAg positive in IVDUs was lower than reported in Greece as 9.8% (16). The absence of HBV-DNA in our IVDUs group is probably indicative of having non reactive virus in the stage of sampling.

The presence of anti-HIV in 8.8% of our IVDUs and none in thalassemics is also indicative of a higher rate of exposure in the first group because of their life style.

Our results indicated that the rate of HCV-RNA and HGV-RNA was similar in thalassemics and IVDUs (Table 2) showing more likely parenteral route of transmission and the fact that these rates are influenced by the actual prevalence of HGV in the population.

In summary, HGV infection occurs with intermediate frequency among thalassemics and IVDUs in Iran. The higher rate of HGV-RNA observed in older IVDUs, suggests a higher exposure of the agent by increasing age or that clearance occurs less frequently once infection is established.

Application of a serologic marker of HGV exposure should document more precisely the incidence and recovery from HGV infection. Additional studies are also needed to clarify the location of HGV replication and the human diseases it causes.

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