

ORIGINAL
ARTICLEHepatitis G Virus Infection in Iranian Blood Donors
and High-Risk GroupsSedigheh Amini Kafi-Abad ^{1*}, Shahram Samiei ², Ali Talebian ³,
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Background and Aims: Hepatitis G virus (HGV) has a worldwide distribution, and the prevalence rates among blood donors and high-risk groups are different. The purpose of this study was to assess the frequency of the HGV infection in blood donors as a blood borne pathogen and in high-risk groups (multitransfused patients), such as thalassemic, hemophilic, and hemodialysis patients.

Methods: 400 Iranian (Tehran Blood Transfusion Center, 2004) blood donors were tested for HGV RNA by a reverse transcriptase chain reaction (RT-PCR) method. The participants were negative in blood screening tests for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti- HCV), human immunodeficiency virus (HIV) Ag/Ab, and Rapid Plasma Reagin (RPR). HGV RNA positivity was surveyed in 40 thalassemic, 16 hemophilic, and 46 hemodialysis patients by RT-PCR. To assess the frequency of infection, the prevalence of HGV RNA positive cases per 100 donors/patients with 95% confidence intervals (95% CI) were calculated. P values were estimated with chi-square tests.

Results: 19 (4.8%; 95% CI: 2.9-6.5%) out of 400 blood donors samples were HGV RNA positive. The prevalence of HGV infection was 5.28% (13 out of 243) in repeat donors, 4.12% (4 in 99) in lapsed donors, and 3.50% (2 out of 58) in first-time blood donors. The combined prevalence of HGV infection in these groups of patients was 16 (15.7%; 95% CI: 8.3-23.1%) out of 102 samples. HGV RNA frequency was 1 out of 40 (2.5%; 95% CI: 1.8-3.2%) thalassemic patients, 15 out of 46 (32.6%; 95% CI: 16.8-48.4%) hemodialysis patients, and 0 out of 16 hemophilic patients.

Conclusions: The prevalence of HGV RNA in the high-risk population was 15.7% and nearly 3 times more than blood donors (4.8%). These data indicate the possibility of parenteral transmission of HGV, especially by transfusion of blood and blood components. Decisions to screen blood supplies for a transfusion-transmitted infection agent should be based on sufficient benefits for recipients.

Keywords: Hepatitis G Virus, Blood Donor, Thalassemia, Hemophilia, Hemodialysis, Iran

Introduction

Hepatitis G virus (HGV) was discovered in 1995. It belongs to the *Flaviviridae* family (1). The detection of HGV viremia is based on the detection of viral RNA in the plasma by the use of polymerase chain reaction (PCR) (2). Antibodies against the envelope protein E₂ of HGV (Anti-E₂), are usually detected after the clearance of the blood from HGV RNA and an enzyme-linked immunosorbent assay method has been developed for its detection (3, 4). HGV is usually transmitted by parenteral and

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vertical routes. Transmission by transfusion of blood and blood products has been established. Sexual transmission has also been suggested (5).

HGV has a worldwide distribution, although the prevalence among blood donors and high-risk groups vary across countries. Anti-E₂ is a recovery marker found in >3% of blood donors and from 13% to 68% in high-risk groups such as intravenous drug abusers (3, 6). The prevalence of HGV RNA has been reported to range from 0.5% to 12.2% in the blood donor population and from 14% to 39% in high-risk patients (3, 7-10). The role of HGV in the pathogenesis of acute and chronic hepatitis, cirrhosis, and liver cancer has not yet been established (5).

The aim of this study was to determine the prevalence of HGV RNA in healthy blood donors and multitransfused patients as recipients of blood or blood components including *Ethalassemia major*, hemophilia and hemodialysis groups in Iran.

Materials and Methods

Blood donors and patients samples

Blood samples from 400 blood donors were collected at a fixed blood collection site in center of Tehran in 2004. All blood donors were voluntary and non-remunerated. The donors were negative for hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV) Ag/Ab, hepatitis C virus antibody (anti-HCV), and Rapid Plasma Reagin (RPR). A first-time blood donor was identified as a donor who donated for the first time and only once. A regular donor was defined as a donor who donated more than once during one year, and a lapsed donor was defined as any donor who had a history of previous donation but the interval between two donations was more than one year.

A total of 102 blood samples from 40 thalassemic, 46 hemodialysis, and 16 hemophilic patients were collected at the Iranian Blood Transfusion Organization (IBTO) references lab. The samples were frozen at -70 °C within 4 hours after collection.

RNA extraction

RNA was extracted from 250 µl of EDTA-anticoagulated plasma using trizol (Invitrogen™) as organic extraction, chloroform, and precipitation with isopropanol-ethanol. Negative (HGV RNA negative plasma) and positive (HGV RNA positive plasma) controls were included in each run.

HGV RT-PCR

An in-house reverse transcriptase PCR (RT-PCR) with primers from NS5a region was used to

detect the specific nucleotide sequence of the HGV genome. The sequence of primers for NS5a gene at position NS5a 77-101 was CTC TTT GTG GTA GTA GCC GAG AGA T, and at position NS5a 211-188 was CGA ATG AGT CAG AGG ACG GGG TAT. The primers were checked by Blast program and were specific for HGV RNA.

Reverse transcriptase was completed during the first step by using HGV- RT mixture, which consisted of a random hexamer (Promega), 20 units of RNase inhibitor (Promega), 20 units of RT-enzyme (MMLV-Promega) and 1 mmol of dNTP (Roche). 5µl of this cocktail was mixed with 5 µl of extracted RNA. The cDNA thermal profile was 25 °C for 10 min, 40 °C for 60 min, and 95 °C for 5 min.

PCR reaction

All 10 µl of cDNA were submitted for PCR reaction by using 20 µl reaction solution containing 10 mmol of tris HCl, pH 8.3, 50 mmol of KCl, 2.5 mmol of MgCl₂, 10% Betaine, 10 µmol of each primer, and one unit Taq polymerase enzyme (Promega). After denaturation at 95 °C for 3 min, DNA fragments were amplified for 45 cycles at 95 °C for 45 seconds, 55 °C for 45 seconds, 72 °C for 45 seconds, and finally 72 °C for 5 min for 1 cycle. The detection limit for this assay was estimated 380 copies/ml.

Detection

The amplified products were visualized after electrophoresis on agarose gels (2%) containing ethidium bromide under ultraviolet light (302 nm).

Statistical analysis

Prevalence rates were calculated at 95% confidence intervals (CI). Chi-square and Fisher's Exact Tests were performed and considered significant if the P value was less than 0.05. The statistical analysis was performed with SPSS (v11.5 SPSS, Inc., Chicago III, USA).

Results

The distributions by age groups, sex, and blood donation status for the 400 blood donors are provided in Table 1. Nineteen out of 400 (4.8%; 95% CI: 2.71-6.89) blood donors' samples were positive for HGV RNA (RT-PCR). The prevalence of HGV infection by age, sex, and blood donor status are shown in Table 2.

In blood donors, the infection rate was higher in 36- to 45-year-olds (8 out of 93, 8.6%) and decreased to approximately 2.5% in the younger age groups

Table 1 Age groups, sex, and blood donation status distributions of 400 blood donors.

Parameters		Age Group (Years)	<25	26-35	36-45	46-55	>56	Total
Sex	Male		76	113	77	63	21	350(87.5%)
	Female		7	8	16	15	4	50(12.5%)
	Total		83	121	93	78	25	400(100%)
Donor Status	First time		23	25	4	5	1	58(14.5%)
	Lapsed		19	29	19	26	6	99(24.8%)
	Repeat		41	67	70	47	18	243(60.7%)
	Total		83	121	93	78	25	400(100%)

Table 2 Age groups, sex, and blood donation status distributions of HGV infection in blood donors.

Parameters		HGV RNA	HGV RNA –	Total	P-value
Sex	Male	16 (4.6%)	334 (95.4%)	350(100%)	0.719
	Female	3 (6.0%)	47 (94.0%)	50(100%)	
	Total	19 (4.8%)	381 (95.2%)	400(100%)	
Age	>25	2 (2.4%)	81 (97.6%)	83(100%)	0.204
	26-35	3 (2.5%)	118 (97.5%)	121(100%)	
	36-45	8 (8.6%)	85 (91.4%)	93(100%)	
	46-55	5 (6.4%)	73 (93.6%)	78(100%)	
	>56	1 (4.0%)	24 (96.0%)	25(100%)	
	Total	19 (4.75%)	381 (95.25%)	400(100%)	
Donor Status	First time	2 (3.5%)	56 (96.5%)	58(100%)	0.771
	Lapsed	4 (4.0%)	95 (96%)	99(100%)	
	Repeat	13 (5.3%)	230 (94.7%)	243(100%)	
	Total	19 (4.75%)	381(95.25%)	400(100%)	

($P = 0.204$). The relative frequency of HGV RNA was higher in repeat blood donors than in first-time blood donors, but this difference was not significant ($P = 0.771$).

HGV RNA was detected in 1 out of 40 (2.5%; 95% CI: 1.8-3.2%) samples in thalassemic patients and 15 out of 46 (32.6%; 95% CI: 16.8-48.4%) samples in hemodialysis patients. None of the 16 hemophilic patients were HGV RNA positive. Overall, HGV RNA prevalence in high-risk population was 15.7% (95% CI: 8.3-23.1%). The distributions of age and sex of the high-risk patients are shown in Table 3.

Discussion

In this study, the prevalence of HGV RNA in Iranian blood donors was reported to be 4.8%, which is slightly higher than several European countries, but

the frequency of HGV viremia in Iran is similar to the prevalence of HGV RNA in most other countries around the world (Table 2). The prevalence of HGV RNA in blood donors around the world is relatively common and ranges from 0.5 to 12.2 % (Table 4). The frequency of HGV RNA in blood donors around the world is estimated to be 4.2%⁽¹¹⁾.

Anti-E₂ prevalence in Iranian blood donors was reported to be 4.2%, so about 9% of Iranian blood donors have been exposed to the virus⁽¹⁵⁾.

The mean age of repeat donors was higher than the mean age of first-time blood donors, and the highest prevalence of HGV RNA was seen in repeat donors (5.35%). In this study the prevalence of HGV viremia was higher in participants aged 36-45 years (42.1%) than in other age groups; this may be a result of increased exposure to HGV at older ages. In several studies, the highest prevalence of HGV RNA was reported for individuals aged 29-39 years.

The prevalence of HGV RNA in high-risk

Table 3 Age groups and sex distributions of 102 patients and the prevalence of HGV RNA in risk groups.

Parameter	Patients Group	Thalassemia			Hemodialysis			Hemophilia	
		HGV RNA -	HGV RNA +	P-Value	HGV RNA -	HGV RNA +	P-Value	HGV RNA -	HGV RNA +
Age									
0-10		5	0	0.664	1	0	0.937	1	0
11-20		20	0		2	0		6	0
21-30		11	1		4	2		6	0
31-40		2	0		9	4		1	0
41-50		1	0		8	5		1	0
51-60		0	0		3	2		0	0
>61		0	0		2	2		1	0
Total		39	1						
Sex									
Male		26	1	1.00	16	10	0.365	16	0
Female		13	0		15	5		0	0
Total		39	1(2.5%)		31	15(32.6%)		16	0 (0%)

Table 4 Prevalence of HGV viremia among blood donors in several countries.

Country	HGV RNA Positive (%)
Japan ^(10, 12)	0.5-1
United States ⁽³⁾	1-1.4
Norway ⁽¹³⁾	2.5
Taiwan ⁽⁹⁾	3.4
France ⁽¹⁴⁾	4.2
Egypt ⁽⁷⁾	12.2

population was 15.7%, nearly 3 times more than blood donors, and this difference was significant ($P = 0.001$). These data indicate a role for parenteral transmission of HGV, probably via blood and blood products ⁽¹⁶⁾.

In regular hemodialysis patients, HGV viremia is persistent for many years, and recovery from it is uncommon ⁽¹⁷⁾. This phenomenon (persistence of viral replication) may be related to the host's immune response and alteration of the host's immunity ^(4, 17). In this study, the prevalence of HGV RNA in hemodialysis patients was 32.6%, and this was higher than the prevalence rates for other high-risk, possibly because of long-term viremia in this group of patients. Eslamifar *et al.* have reported an anti-HGV (anti-E₂) prevalence of 3.98% in 77 hemodialysis patients, and none of their anti-HGV-positive samples were HGV RNA positive ⁽¹⁸⁾.

In previous studies, HGV viremia among hemophilic patients treated with inactivated coagulation factor concentrates was either not reported or was lower than patients treated with non-virus-inactivated clotting factor concentrates ^(4, 19, 20). In this study none of the hemophilic patients were HGV RNA positive, which might indicate that these patients received virus-inactivated clotting factor concentrate. In the Southern Khorasan province (in the northeast) of Iran, the prevalence of HGV RNA in hemophilic patients has been reported to be 5%, and these patients have been found to receive cryoprecipitate more than non-virus-inactivated clotting factor concentrates ⁽²¹⁾.

In the present study, the prevalence of HGV viremia in thalassemic patients was 2.5%. Frequency of anti-E₂ in thalassemia major patients was reported to be 25%, so HGV exposure in this group was estimated at 27.5% but in another study 12.9% of thalassemic patients were HGV RNA positive ⁽²²⁾.

HGV is common in blood donors and in the general population. In comparison, the prevalence rates of HBV, HCV, and HIV are all significantly lower than HGV among Iranian blood donors ($P < 0.000001$) ^(23, 24). HGV can be transmitted by transfusion, and there is a high rate of HGV infection in multitransfused patients. Despite extensive study, HGV has not been identified as a causative agent of liver disease or other known clinical conditions. Decisions to screen blood supplies for a transfusion-transmitted infection agent should be based on sufficient benefits for recipients.

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