

Evaluation of HGV Viremia Prevalence and Its Co-Infection with HBV, HCV, HIV and HTLV-1 in Hemophilic Patients of Southern Khorassan, Iran

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Background and Aims: The GB virus-C (GBV-C) and Hepatitis G virus (HGV), collectively known as GBV-C/HGV and transmitted through blood transfusion and blood components. A co-infection of HGV and HCV is often seen in patients with hemophilia. The paucity of information about rate of GBV-C infection among hemophilic patients in Iran promoted the current study.

Methods: This study was performed on 80 hemophilic patients from south Khorassan branch of Iranian hemophilia society in Birjand. All 80 serum samples were tested for hepatitis B surface antigen (HBs-Ag), Anti HCV, Anti HIV, and Anti HTLV-1. All sera positive for HCVAb were retested by recombinant immunoblot assay as a complementary test. Also, Serum HCV-RNA, HCV genotyping and HGV-RNA were detected.

Results: The prevalence of HGV-RNA was 5% (4 of 80). The prevalence of Anti HCV positive was 26.3% (21 of 80) and HCV- RNA was detected in 80% (17 of 21) of these patients. Co infection of HGV with HCV was 5%. HBsAg and Anti HIV were negative in all of our patients. Anti HTLV-1 was detected in one patient (1.25%).

Conclusions: HGV and HCV are prevalent in South Khorassan hemophilic patients. Prevalence of HGV infection is less than HCV but it is more prevalent than HBV, HIV and HTLV-1 infection.

Keywords: GBV-C, Hemophilia, Hepatitis G, Hepatitis C

Introduction

GB virus-C/hepatitis G virus (GBV-C/HGV) is an envelope positive-stranded RNA virus with a genome of about 9.4 kb belonging to the Flaviviridae family. It is distributed worldwide, and at least five major genotypes of this virus have been proposed based on sequence analysis of the 5' noncoding region 5' (NCR) or E2 gene ⁽¹⁻³⁾. GBV-C/HGV is transmitted through blood transfusion and blood components ⁽⁴⁾. Epidemiological data suggest that this virus can also be spread by sexual or vertical transmission ^(5, 6); however, little is known about other ways of transmission that could explain its high prevalence and worldwide distribution. Different modes of transmission have been proposed to explain the ubiquitous presence of this virus; for example, a high prevalence (22 to 38%) of viral RNA has been reported in populations with parenteral risk ^(7, 8). However, similar prevalence rates have also been observed in groups with little or

no parenteral risk, such as blood donors ⁽⁷⁻¹⁰⁾. Most HGV infections appear to be asymptomatic. A few cases of fulminant hepatitis have been reported, but it remains unclear whether the hepatitis was actually caused by HGV. Anti-E2 seroconversion seems to be associated with viral clearance. A co infection of HGV with HCV, often seen in patients with hemophilia, nevertheless, seems not to affect the course of HCV infection or the response to interferon-alpha therapy ^(11, 12).

Hemophilic patients are considered as high-risk

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group for blood born diseases, due to receiving blood and blood components. To date, there is no data on prevalence of HGV in hemophilia patients in south Khorassan state in Iran. The purpose of our study was to evaluate the prevalence of HGV infection in hemophilic patients and also to estimate its co-infection with HBV, HCV, HIV and HTLV-1 infection.

Materials and Methods

This study was performed on all hemophiliacs registered (80 patients) in Iranian hemophilia society in Birjand. Venous blood samples were taken from each patient, and the serum samples were stored at -80°C until use.

Patients

Blood samples were taken from 80 patients born between 1946 and 2003. 88.75% of these patients had hemophilia A, 2.5% had hemophilia B, 2.5% vonWillebrand disease, and the rest had other types of factor deficiency diseases (Table 1). All samples were tested for HGV-RNA, anti-HCV, anti-HBV, anti-HIV, and anti-HTLV-1.

Table 1. Distribution of our patients regarding the type of hemophilia or hemorrhagic disorder

Type of hemophilia	Frequency	Percent
Hemophilia type A	71	88.75
Factor II deficiency	3	3.75
vonWillebrand disease	2	2.5
Hemophilia type B	2	2.5
Factor V deficiency	1	1.25
Factor XIII deficiency	1	1.25
Total	80	100

Transfusion History

Based on the viral safety and the origin of the clotting factor concentrates, three categories of products can be distinguished: Group A, nonviral inactivated small pool cryoprecipitate or nonviral inactivated large pool clotting factor concentrate (used until 1985); group B, small pool cryoprecipitate (1985 to 1992) or large pool clotting factor concentrate (1985 to 1990), which were suboptimal viral inactivated (dry heating of lyophilized products at 60°C for 72 hours); group C, large pool optimal viral inactivated clotting factor concentrate (SD treated, pasteurized) (1990 to present) or recombinant DNA-derived clotting factor concentrate (1992 to present) ⁽¹¹⁾. Due to this

history of blood products, we classified our patients regarding the year of birth into 3 groups: group 1 consisted of 39 Patients born before 1985, group 2 included 23 patients born between 1986-1995, and group 3 included 18 patients born between 1996-2003. In Iran all blood products have been screened for HCV since 1996 ⁽¹³⁾.

Assays

All 80 serum samples were tested by commercially available enzyme-linked immunosorbent assay (ELISA) kits to detect anti-HCV antibodies (HCVAb) (Hepanostika® HCV ultra, China), hepatitis B surface antigen (HBsAg) (Enzygenost® HBS Ag, USA), anti-HIV antibodies (Genscreen HIV; Bio Rad®, France) and anti-HTLV-1 antibodies (Gene labs® Diagnostics HTLV-I/II, Switzerland). All sera positive for HCVAb were retested by the second generation of recombinant immunoblot assay (RIBA) kits (Diagnostics® HCV blot; Germany) as a complementary test. Serum HCV-RNA was detected by a nested reverse transcription RT-PCR (Diasorin® HCV-RNA, Spain) and HCV genotyping was performed by direct sequencing of the 5' noncoding region. HGV-RNA was detected by nested RT-PCR (Diagnostic® HGV-RNA, U.S.A) using primers targeting the 5' - untranslated region (UTR).

Statistical analysis

Difference in the recovery rate of HGV and HCV infection was analyzed with chi-square for independence and Fisher's exact probability test. A P value <0.05 was considered significant.

Results

Mean age of our patients was 21.30 ± 12.11 years. Seventy-seven patients were male (96.3%) and (3.8%) female. 88.75% of the patients had hemophilia A (Table 1). Classification of these patients regarding severity of hemophilia showed that 11 patients (15.5%) had mild hemophilia, 50.7% moderate hemophilia, and 33.8% severe hemophilia. Our results indicated a significant relation between HCV infection with the severity of hemophilia ($P=0.044$).

Virological findings

In all the patients, the prevalence of HGV-RNA was 5%. The prevalence of Anti HCV positive was 26.3% (21 of 80) and HCV-RNA was detected in 80% (17 of 21) of these patients. The prevalence of HCV viremia (21.3%) was much higher than that

of HGV viremia. Co-infection of HGV with HCV was 5% (1 of 21). Results of genotyping in 17 patients with positive HCV-RNA were as follows: 59% HCV genotype 3a, 35% HCV genotype 1a and 6% HCV genotype 2. HBsAg and Anti HIV were negative in all of our patients. Anti-HTLV-1 was detected in one patient (1.25%) with ELISA method and confirmed by recombinant immune blot assay HTLV-1 test. In addition HCV-RNA and HGV-RNA in this patient were negative (Figure 1).

Cross tabs results

There was a significant relation between age and anti-HCV positive state in our patients ($P=0.007$), (Table 2). We didn't find a significant relation between sex of the patients and positive anti-HCV state ($P=0.29$).

Discussion

This study showed that 5% of our patients were HGV-RNA positive. Other studies reported the

prevalence of HGV infection among hemophilic patients ranging from 9% to 48% (14-21). Prevalence of HGV infection was less than that of previous studies done on hemophilia patients in other countries and also less than HGV infection rate in thalasemic patients (12.9%) in Iran that was reported by Amini *et al.* (22). Some hypotheses may explain the lower prevalence of HGV infection among our patients. First, our patients have been received lower amounts of factor VIII concentrates because our region is far from the capital and most of our patients live in rural regions and as a result, they receive less factor VIII concentrates. Second, when factor VIII is less available in Iran, our patients received cryoprecipitate instead, and because cryoprecipitate is prepared from blood of local blood donors with lower prevalence of blood borne diseases such as AIDS, HCV, and HBV infection in our area (none of our patients were HBsAg or HIV positive), patients encountered lesser infected blood products. Consequently, the incidence of HGV infection like other blood borne diseases was much less.

Furthermore, our study showed that Anti HCV was positive in 26.3% (21) of our patients and (80%) patients from this group was HCV-RNA positive. Prevalence of HCV infection was reported 98.6% in Germany hemophilic patients (19), 54.5% in Indian hemophilic patients (23) and 60.2% in hemophilic patients in Iran (24).

10-20% of HCV patients are co-infected with HGV (25). At least this can be due to one common way of transmission. In a study this co-infection was reported to be about 40% in high risk population in Iran (22). In our study this co-infection was observed in nearly 5% (1 of 21) hemophilic patient. The low prevalence of the co infection is in accordance with low prevalence of other blood borne diseases in the region.

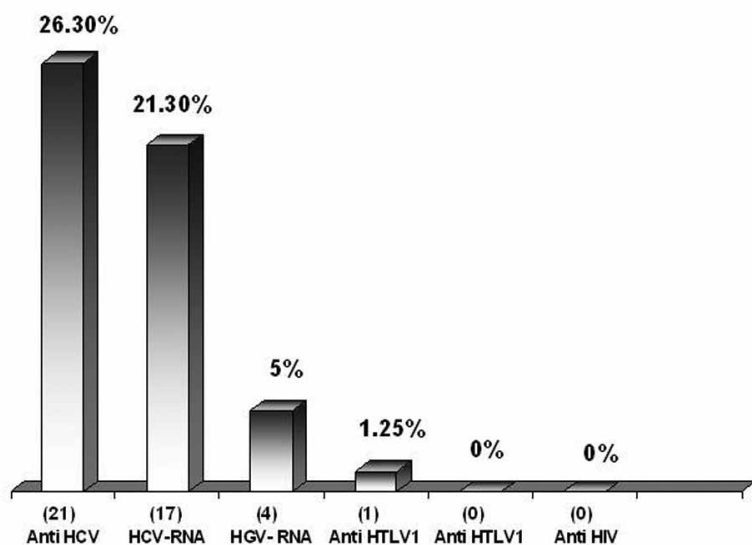


Figure 1. HGV viremia and the presence HCV viremia and serologic markers of other blood borne diseases

Table 2. Frequency of Anti HCV positive state versus year of birth ($P=0.007$)

		Anti-HCV		Total	
		Positive	Negative		
Classification of birth year	Before 1985	Count/Percent	11 (52.5%)	28 (47.5%)	39 (48.8%)
	1985-1995	Count/Percent	10 (47.5%)	13 (22%)	23 (28.7%)
	After 1995	Count/Percent	0 (0%)	18 (30.5%)	18 (22.5%)
Total		Count/Percent	21 (100%)	59 (100%)	80 (100%)

Besides, we found a significant relation between age and HCVAb positive state ($P=0.007$) in which most positive HCVAb state was seen in those born before 1995 but we didn't find any significant relation between sex and HCVAb positive state ($P=0.29$) (Table 2).

Evelin *et al.*, found a similar relation between HCV RNA positive state and age ($P=0.001$) and those who were HCV RNA positive were mostly born between 1960-1969. They found also a significant relation between HGV RNA positive state and age ($P=0.026$) with greater prevalence of positive HGV RNA state in those born between 1980-1989⁽¹¹⁾.

Conclusion

HGV and HCV are prevalent in hemophilic patients of south Khorassan, Iran. Prevalence of HGV infection is less than HCV, although it is more prevalent than HBV, HIV and HTLV1 in them. As this virus can be transmitted by blood and its products, it is recommended to study the viremia and clinical manifestations in other centers too.

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