Prevalence and Genotypes of GB Virus C/Hepatitis G Virus among Blood Donors in Central Brazil

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A survey was conducted in a blood donor population of Central Brazil aiming to investigate the prevalence of GB virus C (GBV-C)/hepatitis G virus (HGV) infection and also to analyze the virus genotypes distribution. A total of 241 voluntary blood donors were interviewed at the State Blood Bank in Goiânia, State of Goiás, Brazil. Blood samples were collected and serum samples tested for GBV-C/HGV RNA by polymerase chain reaction. Genotypes were determined by restriction fragment length polymorphism (RFLP) analysis. Seventeen samples were GBV-C/HGV RNA-positive, resulting in a prevalence of 7.1% (95% CI: 4.2-11.1). A significant trend of GBV-C/HGV RNA positivity in relation to age was observed, with the highest prevalence in donors between 29-39 years old. Ten infected individuals were characterized by reporting parenteral (30%), sexual (18%), both (6%) and intrafamiliar (6%) transmission. However, 7 (40%) GBV-C/HGV RNA-positive donors did not mention any potential transmission route. RFLP analysis revealed the presence of genotypes 1 and 2 of GBV-C/HGV; more precisely, 10 (58.9%) samples were found belonging to the 2b subtype, 4 (23.5%) to the 2a subtype, and 3 (17.6%) to genotype 1. The present data indicate an intermediate endemicity of GBV-C/HGV infection among this blood donor population, and a predominant circulation of genotype 2 (subtype 2b) in Central Brazil.

Key words: hepatitis G virus - GB virus C - blood donors - Goiânia - Central Brazil

GB virus C (GBV-C) and hepatitis G virus (HGV) are independent isolates of the same virus which were identified as possible aetiological agent of viral hepatitis in humans (Simons et al. 1995, Linnen et al. 1996). The GBV-C/HGV can cause persistent infection, but its role in causing liver diseases is still uncertain (Bowden 2001). However, some studies showed that infection with GBV-C/HGV can be associated with lower progression of human immunodeficiency virus (HIV) disease in coinfected patients (Lefrère et al. 1999, Yeo et al. 2000, Tillmann et al. 2001, Xiang et al. 2001).

The GBV-C/HGV genome consists of a single-stranded positive sense RNA of approximately 9.4 kb which has characteristics of a flavivirus-like genome as in the case of the hepatitis C virus (HCV) (Muerhoff et al. 1995), except that unlike HCV, the 5' noncoding region (5' NCR) of GBV-C/HGV is variable, and can be used initially to classify natural isolates into three genotypes. Genotype 1 is frequently found in West Africa, genotype 2 predominates in the USA and Europe, and genotype 3 is commonly observed in parts of Asia (Muerhoff et al. 1996, 1997, Mukaide et al. 1997, Okamoto et al. 1997, Katayama et al. 1998). In addition to this classification, 2 novel genotypes were identified. Genotype 4 was described in Myanmar and Vietnam (Naito et al. 2000), and genotype 5 has been found in South Africa (Tucker et al. 1999, Tucker & Smuts 2000).

GBV-C/HGV is transmitted through blood transfusion and blood components (Schmidt et al. 1996, Roth et al. 1997, Heuft et al. 1998). Epidemiological data suggest that this virus is also spread by sexual and vertical transmission (Bourlet et al. 1999, Stark et al. 1999, Wejstål et al. 1999). However, little is known about other modes of transmission that could explain the high prevalence and worldwide distribution of this virus.

In Brazil, high GBV-C/HGV RNA prevalence rates were found in blood donors from Southeast and Northeast regions (Bassit et al. 1997, Lampe et al. 1998a, Goubau et al. 1999, Pinho et al. 1999). As data concerning GBV-C/HGV infection in other Brazilian regions are still rare, we sought to assess the prevalence of GBV-C/HGV RNA in blood donors in Central Brazil and also to investigate the virus genotypes distribution.

MATERIALS AND METHODS

Subjects - From June to September 2000, a total of 241 voluntary blood donors accepted for blood donation, after clinical evaluation, at the State Blood Bank in Goiânia, State of Goiás (1,000,000 inhabitants), Central Brazil, were invited to take part of this study, and informed consent was obtained from all participants. The study was approved by the Ethical Committee of the Federal University of Goiás.

A standardized form was used to collect sociodemographic and data as number of previous blood transfusions, acupuncture, tattooing, surgery, intravenous drug use, dental procedure with non-licensed dentist, multiple sex partners, sexually transmitted diseases, and possible household contact with hepatitis.

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Serological tests - Blood samples were collected from all donors and sera were stored at -20ºC. They were screened for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc) and hepatitis C antibody (anti-HCV) by enzyme-linked immunosorbent assays (ELISA) (Abbott Laboratories, USA). All samples were also tested for alanine aminotransferase (ALT) levels by a colorimetric method (Dolles Laboratory, Brazil).

Detection of GBV-C/HGV RNA - All samples were submitted to RNA extraction, reverse transcription, and a nested polymerase chain reaction (PCR) with primers complementary to the conserved area of the NS5 region of the genome, essentially as described by Lampe et al. (1997).

GBV-C/HGV genotyping - GBV-C/HGV RNA-positive samples were amplified by PCR using primers complementary to the 5' non-coding region (NCR). Genotypes were determined by means of RFLP method (Quarleri et al. 1999). Briefly, amplicons were initially cleaved with *Hin*f I, and depending on the restriction pattern observed, a second digestion was performed either with *Aci* I or *Aat* II. Restriction fragments were resolved in ethidium bromide-stained 3% agarose gels.

Statistical analysis - Prevalence and 95% confidence intervals (CI) were calculated. Chi-square test, Chi-square for trend test or Fisher's exact test were performed to evaluate the distribution of characteristics associated with GBV-C/HGV infection. Statistical significance was assessed at the 0.05 probability level in all analyses. Statistical evaluations were performed using Epiinfo 6.0 program developed by the Centers for Disease Control and Prevention (Atlanta, GA).

RESULTS

The studied population ranged in age from 18 to 60 years (mean ± SD = 30.2 ± 8.5 years). The majority of the blood donors (86.7%) were men. Almost 70% of them earned less than U$ 200 per month and also had less than 8 years of schooling. Forty-eight (19.9%) were first-time blood donors and 193 (80.1%) were regular donors. This population consisted of relatives or close friends of hospitalized patients needing blood transfusions.

As shown in Table I, a prevalence of 7.1% (95% CI: 4.2-11.1) was found for GBV-C/HGV infection. This donor population showed positivity rates of 5% and 0.8% for hepatitis B (anti-HBc) and C (anti-HCV), respectively. All serum samples were negative for HBsAg.

Analysis of the characteristics of this population showed that only age was significantly associated with GBV-C/HGV infection. This infection reached a peak at an age range from 29 to 39 years old; 13.5% of the blood donors were infected. Moreover, 2.5% of the age group under 28 years and 6.1% of age group over 40 years were GBV-C/HGV RNA- positive (χ^2 for trend = 9.98; p = 0.018). On the other hand, potential transmission routes associated with parenteral (30%), sexual (18%), both (6%) and intrafamiliar (6%) were reported by GBV-C/HGV RNA-positive donors. However, 40% of them did not mention any known source for infection (Table II). GBV-C/HGV RNA-positive individuals ranged in age from 21 to 43 years. All but one were men. The majority of them were regular

TABLE I

Detection of GB virus C/hepatitis G virus RNA and serological status for hepatitis B and C among 241 blood donors in Central Brazil

Viral markers	Positive		95% CI
	n	(%)	
Anti-HBc	12	(5)	2.6-8.5
Anti-HCV	2	(0.8)	0.1-3.0
GBV-C/HGV RNA	17	(7.1)	4.2-11.1

CI: confidence interval

TABLE II

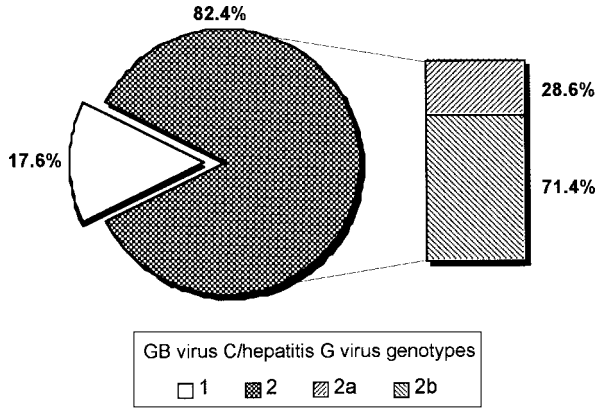
Characteristics of 17 GB virus C/hepatitis G virus RNA-positive blood donors

Donor no.	Age (yr)	Gender	Donation	Possible transmission route	Anti-HBc	ALT
1	43	F	First	Surgery	-	15
2	31	M	Regular	-	-	10
3	36	M	Regular	-	-	17
4	37	M	Regular	Dental procedure [a]	-	26
5	39	M	Regular	-	+	28
6	28	M	Regular	STD	-	15
7	21	M	Regular	Multiple partners	-	14
8	32	M	Regular	STD	-	6
9	34	M	Regular	Multiple partners	-	17
10	37	M	Regular	-	-	23
11	43	M	Regular	Dental procedure	-	20
12	29	M	Regular	Household contact [b]	-	26
13	31	M	First	Transfusion, tattoo, surgery	-	10
14	25	M	Regular	Tattoo	+	28
15	36	M	Regular	-	-	8
16	38	M	Regular	-	-	23
17	32	M	Regular	-	-	21

a: history of dental procedure with non-licensed dentist; *b*: possible household contact with hepatitis; ALT: alanine aminotransferase; STD: sexually transmitted disease

blood donors. Two of these donors (nos. 5 and 14) were anti-HBc positive. All GBV-C/HGV infected individuals had normal ALT levels.

All 17 GBV-C/HGV RNA-positive samples were genotyped by RFLP pattern. It was observed that 3 (17.6%) were of genotype 1 and 14 (82.4%) of genotype 2. Of these, 4 isolates belonged to the 2a subtype and 10 to the 2b subtype (Figure).



Frequency of GB virus C/hepatitis G virus genotypes in blood donors in Central Brazil.

DISCUSSION

Epidemiological investigations demonstrated that GBV-C/HGV RNA prevalence among blood donors ranges from 0.5-4% in the USA, Europe and Japan (Orito et al. 1996, Gutierrez et al. 1997, Nübling et al. 1997, Blair et al. 1998, Mercier et al. 1999, Sauleda et al. 1999) to 10-18.9% in some African countries (Casteling et al. 1998, El-Zayadi et al. 1999, Sathar et al. 1999). In South America, it has also been reported at a varying range from 5.5% in Argentina (Oubiña et al. 1999) to 14.6% in Bolivia (Konomi et al. 1999). In Brazil, rates of 9% and 10% were detected in blood donors in São Paulo and Rio de Janeiro (Southeastern region), respectively (Bassit et al. 1997, Lampe et al. 1998a). In addition, 5.2% and 6.5% of donors with normal and elevated ALT levels were GBV-C/HGV RNA-positive (Pinho et al. 1999). A prevalence of 8.6% was found in Fortaleza (Northeastern region) (Goubau et al. 1999). Thus, the prevalence of 7.1% found in blood donors in Goiânia could be placed in an intermediate position.

The highest prevalence of GBV-C/HGV RNA was seen in the group from 29 to 39 years (13.5%). A similar finding of age-specific prevalence has been reported by Konomi et al. (1999), with a peak at an age range from 20 to 39 years in healthy Bolivian individuals. In addition, among 170 blood donors in Rio de Janeiro, the seroprevalence of antibody against GBV-C/HGV envelope E2 protein increased with age, from 5.6% (group with ages between 18 and 24 years) to 35.3% (donors from 43 to 60 years) (Lampe et al. 1998b). These data may suggest a role for sexual transmission of GBV-C/HGV.

In the present study, prevalence rates of 5% and 0.8% were found for hepatitis B and C, respectively. Of the 17 GBV-C/HGV RNA-positive blood donors, two were positive for anti-HBc, but negative for HBsAg, and none of them was coinfected by HCV. In addition, no association was found between GBV-C/HGV RNA status and serum ALT levels. These data indicate that this virus has been independently widespread in healthy individuals in Central Brazil.

We observed the characteristics of the GBV-C/HGV RNA-positive donors. Ten individuals had exposure to one or more factors associated with parenteral (30%), sexual (18%), both (6%) and intrafamiliar (6%) transmission. However, it is interesting to note that 40% of the infected donors did not mention any identifiable source of infection, but they earned less than US$ 200 per month and had a low level of education. Thus, low socioeconomic status and poor hygienic conditions occurring in developing countries may contribute to GBV-C/HGV dissemination (Konomi et al. 1999).

To analyze the genetic diversity of GBV-C/HGV isolates among blood donors in Central Brazil, all GBV-C/HGV RNA-positive samples were genotyped by RFLP. Three samples (17.6%) belonged to genotype 1 and the remaining 14 (82.4%) to genotype 2. These data demonstrate the simultaneous circulation of both genotypes, already reported to be predominant in West Africa (genotype 1) and USA/Europe (genotype 2) (Tucker & Smuts 2000). Recently, the same genotypes were detected in 35 serum samples in a Brazilian rural population (Northeastern region); more precisely, 82.9% and 17.1% were characterized as genotypes 2 and 1, respectively (Gallian et al. 1998). Also, Lampe et al. (1998b) showed that 31 isolates from individuals living in Rio de Janeiro (Southeastern region) belonged to three clusters, 2 of which were classified as genotypes 1 and 2. The presence of both genotypes in Brazil is likely to reflect the European and African origin of the population. In the present study, genetic diversity of genotype 2 revealed that 4 donors were infected with subtype 2a and 10 with subtype 2b. In Argentina, genotype 2 was also predominant among blood donors, and subtypes 2a and 2b were equally detected (Oubiña et al. 1999). Genotype 3 was not observed in Brazilian isolates in spite of immigration from Asian countries, but it was found in other populations of South America, such as native Indians from Colombia (Tanaka et al. 1998) and in Bolivians (Konomi et al. 1999).

In conclusion, our data point out an intermediate endemicity of GBV-C/HGV infection in Central Brazil, and that the parenteral route was the presumed means of virus transmission for only one-third of the infected blood donors. This investigation also demonstrates the simultaneous circulation of genotypes 1 and 2, with a high prevalence of subtype 2b of GBV-C/HGV in the study population.

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