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High prevalence and autochtonous transmission of human pegivirus (HPgV-1) in blood donors in the extreme southern of Brazil

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Short title: HPgV-1 in blood of donors of southern Brazil

ABSTRACT

Recent studies have suggested that human pegivirus 1 (HPgV-1) may have some pathogenic potential. In the southernmost region of Brazil, studies on HPgV-1 are scarce, and circulating genotypes have not yet been identified. The present study aimed to evaluate the prevalence of HPgV-1 among blood donors from the southernmost region of Brazil, and identify the genotypes involved with associated factors. A cross-sectional study was conducted with 281 blood donors, who had their plasma subjected to RNA extraction, cDNA synthesis, HPgV-1 detection by *nested*-PCR, and subsequent genotyping. The observed prevalence of HPgV-1-RNA was 21.7%. The only variable that was significantly associated with virus infection was the relationship status of the donor. Single or no fixed partner blood donors were twice as likely to have HPgV-1 (95% CI 1.12-4.56, p = 0.02). Genotype 2 - subtypes 2b (69%) and 2a (29%) - was the most prevalent. In the absence of risk factors for parenteral transmission, it is likely that sexual transmission was the route of infection in the individuals studied. Further work will be needed to determine whether this virus is inert in the population, or if there are a potential deleterious effects in infected individuals.

Keywords: RNA extraction; Flavivirus; Blood.

INTRODUCTION

Human pegivirus1 (HPgV-1), formerly known as GB virus type C (GBV-C), was identified in 1960 in a patient with acute hepatitis of unknown cause and was considered a new cause of non-A-E viral hepatitis[1].

HPgV-1 was subsequently found to not cause hepatitis and to replicate in peripheral blood mononuclear cells *ex vivo*. However, the major

in vivo cell targets of HPgV-1 have not yet been fully unveiled. In chronically infected subjects, the virus is found in natural killer, T- and B-cells, and in monocytes, suggesting a tropism of the virus to those cells[2].

HPgV-1 is an RNA virus of the Flaviviridae family, more recently attributed to the *Pegivirus* genus[3,4]. To date, seven genotypes have been found (1 to 7). HPgV-1 infection has a worldwide occurrence. Genotypes 1 and 2 are prevalent in North and Central America and in Africa; genotypes 3 and 4 are common in Asia; while genotypes 5, 6 and 7 are found in Central and Southern Africa, Southeast Asia and China, respectively[5-7]. Infection of the same individual with multiple genotypes and recombination of HPgV-1 are also possible[8,9]. In Brazil, genotypes 1, 2, and 3 have been found in selected populations as blood donors and individuals infected with the human immunodeficiency virus (HIV)[10-15]. However, studies on this subject are scarce and the circulating strains have not yet been mapped in the extreme southern Brazil.

HPgV-1 can be transmitted through parenteral, sexual or mother-to-child routes[16-19]. Co-infection with HIV or hepatitis C virus (HCV) are common due to shared transmission modes, and also in those exposed to transfused blood products, transplanted patients, injection drug users, hemophiliacs, and patients requiring hemodialysis[20]. In the general population, the prevalence of HPgV-1 ranges from 1–4% in North America and Europe, and from 5–19% in Africa, Asia and South America[20]. In Brazil, the prevalence of HPgV-1 in blood donors and healthy volunteers is

estimated between 5.1% and 9.7%, with circulating genotypes 1 and 2[10,21,22].

To date, no epidemiological studies have been carried out on blood donors in the extreme southern region of Brazil to identify the prevalence and circulation of HPgV-1. Because it is considered non-pathogenic, screening for this viral agent is not routinely performed in blood banks. Screening is also avoided due to its considerable prevalence in the general population, which could have the potential to restrict the number of donors. In this scenario, the current study aimed to evaluate, for the first time, the prevalence and circulating genotypes of HPgV-1, and associated factors, in blood donors from the southernmost region of Brazil.

MATERIALS AND METHODS

Ethical aspects

This study has been approved by the Health Research Ethics Committee of Associação de Caridade Santa Casa do Rio Grande (ACSCRG) (n° 23/2015). All participants signed a written informed consent to be enrolled in the study.

Study design and patients

This is a cross-sectional study to evaluate the prevalence and genotypic circulation of HPgV-1 and associated factors in blood donors in the city of Rio Grande, RS, in the southernmost region of Brazil. All participants were 18 years of age or older and were approved by the

Hemotherapy Service of ACSCRG for blood donation after initial screening, and were found to be negative for HIV, HBV and HCV infection as part of the standard blood donation screening. Sociodemographic and behavioral data were collected using a structured questionnaire. Interviews and collection of blood samples occurred between December 2016 and February 2017. Sample size was calculated using Epi-Info v.3.5.2 software (CDC, Atlanta, USA), based on a prevalence of 5% to 10% HPgV-1 among blood donors[10,21,22].

RNA extraction

Virion RNA was extracted from 140µL of plasma with the QIAamp Viral RNA extraction kit (QIAGEN) following the manufacturer's specifications. RNA was eluted in a final volume of 60 µL of the elution buffer and stored at -70°C before complementary DNA (cDNA) synthesis.

cDNA synthesis

Ten microliters of the eluted RNA were added to 300 ng of N_6 random oligonucleotides (2 µL of a solution at 150 ng/µL; Life Technologies, Carlsbad, CA), and denatured for 10 min at 70°C. A reaction using 200U reverse transcriptase (Superscript; Gibco), 0.1M of DTT, 5U of RNaseOUT 1 (Life Technologies), and 0.5mM of each desoxinucleotide was carried out in a final volume of 20 µL at 42°C for 1.5 hr for cDNA synthesis. From the synthesized cDNA, the HPgV-1 non-coding genomic region 5'NCR was PCR-amplified as described below.

Nested PCR for HPgV-1

To detect HPgV-1 in the plasma from blood donors, a version adapted from the PCR protocol described by Jarvis et al.[23] was followed. PCR was performed using two primer pairs in a nested assay. The first round was performed with 5 µL of the obtained cDNA and the second with 5 µL of the product of the first PCR. Both reactions used 1x PCR Buffer, 2mM $MgCl_2$, 0.5mM dNTPs, 1U of recombinant Taq DNA polymerase enzyme (Invitrogen), Mili-Q water q.s.p. to a final volume of 50 µL and 0.5 µM of primers HGV1 and HGV2 (first round) or HGV3 and HGV4 (second round). Primers sequences were as follows: HGV1 forward 5'-AGGTGGTGGATGGGTGAT3'; HGV2 reverse 5'-TGCCACCCGCCCTCACCCGAA-3'; HGV3 forward 5'-TGGTAGGTCGTAAATCCCGGT-3'; HGV4 reverse 5'-GGAGCTGGGTGGCCCCATGCAT-3'. Using those primers, a 344-bp PCR product is expected from the 2nd round reaction. PCRs were performed in a thermocycler with the following cycling: an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, and a final extension step at 72ºC for 2 min. PCR products from the second round were on a 1.5% agarose gel electrophoresis. For this, 5 µL of the PCR product from each sample were mixed with 1 µL of Blue Green LoadingDye (LGC Technology). Gels were visualized on a UV transilluminator and images were captured. A positive control for HPgV-1 (providing a PCR band of 344 bp), confirmed by direct sequencing of the

PCR product, and an HPgV-1-negative sample were used, in addition to a blank reaction containing no DNA sample.

HPgV-1 genotyping

For the HPgV-1 genotyping and phylogenetic analysis, PCR products were purified using the *GFX PCR DNA and TM-Gel Band Purification* kit (GE Healthcare, São Paulo, Brazil) and had their DNA sequence determined suing the *ABI PRISM 1 BigDye™ Terminator Cycle Sequencing Ready Reaction* kit (Life Technologies). DNA sequencing was carried out in an ABI 3130xl Genetic Analyzer (Life Technologies). Chromatograms were edited using SeqMan (DNAStar, Madison, WI). Sequence alignment was conducted using CLUSTALW implemented in BioEdit[24]. Consensus sequences were converted into FASTA format and aligned with reference sequences of HPgV-1 genotypes retrieved from GenBank. Aligned sequences were subjected to phylogenetic analysis with the neighbor-joining method, the Kimura two-parameter model and 1,000 bootstrap replicates using MEGA 5[25] to infer HPgV-1 genotypes. The HPgV-1 sequences described were submitted to GenBank and were assigned the accession numbers MH000531 to MH000584.

Statistical analysis

Sociodemographic and behavioral data variables were analyzed. The chi-square test was employed for comparing categorical variables. Prevalence ratios and potential risk and protective factors were calculated, and frequency and percentages were complied. Differences were

considered statistically significant when $P < 0.05$. Multivariate analysis using Poisson regression was additionally carried out, followed by construction of a hierarchical linear model that incorporated variables with $P \leq 0.20$ in the previous analysis. Socioeconomic variables were considered in the first level, while behavioral variables were used in the second level. All analyses were conducted using SPSS for Windows v.12 (IBM Corp., Armonk, N.Y.) and Epi-info v.3.5.2 (Centers for Disease Control and Prevention).

RESULTS

Samples were obtained from 281 blood donors. The prevalence of HPgV-1 RNA was 21.7% (n = 61). On average, the study population was 33.6 years old (SD = 11.36), participants had completed 10.9 years of study (SD = 3.58), and had 1.5 sexual partners per year (SD = 2.12). The socioeconomic and behavioral characteristics of the donors are presented in Table I. After bivariate analysis of socioeconomic risk factors, ethnicity was the only variable found to be significant (95% CI 0.92-4.46, P = 0.05); however, this variable was not statistically significant after adjustment for other variables. Thus, socioeconomic risk factors showed no overall statistically significant association with HPgV-1-RNA infection. Interestingly, although not significant, an association of infection with age could not be ignored. Donors aged between 18 and 50 years were more frequently infected by HPgV-1 (93.4%, 57 individuals) than those aged ≥ 51 years (6.6%, 4 individuals) (95% CI: 0.69-4.53, $P = 0.20$, Table I). The only significant behavioral variable associated with the presence of HPgV-1

RNA was relationship status. Blood donors who were single or did not have a fixed partner were twice as likely to be infected with the virus as those who were married or had a fixed partner (95% CI 1.12-4.56, *P* = 0.02; Table II). Regarding sexual activity, there were no statistically significant trends, although a higher prevalence of HPgV-1 was observed in those who had more than four sex partners per year. The “tattoo” variable was significantly associated with HPgV-1 infection in the bivariate analysis, but it lost significance after adjustment for other variables (95% CI: 0.96-3.05, *P* = 0.07; Table II).

HPgV-1 subtypes were determined in 52 healthy blood donor subjects who were positive for the virus (85%) by phylogenetic inference. The most prevalent genotype was genotype 2, with subtypes 2b (69%, n = 36) and 2a (29%, n = 15) represented (Figure 1). Genotype 1 was found in only one individual (2%). Despite the low number of positive and genotyped samples, an attempt was made to cross subtypes 2a and 2b (in greater number) separately as dependent variables but no association was observed with the independent variables studied (data not shown). All remaining variables studied were non-significant.

DISCUSSION

The present study was the first evaluation of the prevalence of HPgV-1 in blood donors and the first time that the circulating strains of this virus have been identified in the southernmost region of Brazil. The prevalence of HPgV-1 infection in blood donors in this region was 21.7%.

This is expected when compared to the prevalence of HPgV-1 RNA in blood donors from other developing countries such as South America, Africa, and Asia, where rates vary from 5% to 19%[20]. Regarding blood donors in Brazil, existing studies estimate the prevalence of HPgV-1 to be between 5% and 10%[10,21,22]. Our findings suggest that the rate of infection among blood donors may be higher in the southern region than in other regions of the country. An important issue to consider when using PCR to determine the prevalence of an infectious agent is the choice of amplification target. The 5'NCR region of HPgV-1 was chosen because it is well conserved among isolates and easy to amplify. The same region was used in all previous Brazilian studies that involved blood donors[10,21-22]. Therefore, our findings likely reflect a true local condition in the southernmost region of the country, rather than a difference in the target gene region.

Of the sociodemographic and behavioral variables studied here, only relationship status showed a significant association with HPgV-1 infection. Also, the majority of people infected with HPgV-1 were aged between 18 and 50 years (93.4%); only 6.6% were aged ≥ 51 years. It is known that HPgV-1 can persist as a chronic infection in about 20 to 30% of healthy people, but most eliminate the virus within a few years of the initial infection. A study by Da Mota et al[26] suggested that the risk of HPgV-1 infection decreases with age. Another issue to consider is that younger persons are usually more sexually active, which may increase the likelihood

of HPgV-1 acquisition. Sexual activity decreases with age, thus diminishing the risk of infection[21,27].

In the present study, parenteral transmission was not found to be a significant risk factor for HPgV-1 infection. It is possible that some donors may have omitted certain risk practices for parenteral transmission so their blood was not discarded after screening, since reporting previous syringe sharing, tattoos or transfusions within 12 months of donation are exclusion criteria. In the face of any patient surgery, his/her relatives are asked to recruit donors to replenish blood supplies, and that may influence the veracity of the responses from donors in order to complete the number requested. This may impose a limitation to the present study.

Yet parenteral transmission is the most well documented and considered effective, notably via blood and blood products[17], sexual transmission of HPgV-1 has been recognized[28]. In a study conducted in São Paulo, of HPgV-1 sexual transmission was determined as very efficient[21]. The virus prevailed among sexually active individuals, particularly among those involved in high-risk sexual practices, like in men who have sex with men[21]. Sexual transmission appears to be a major route of HPgV-1 infection, and this could be the reason why relationship status is the only variable that presented an increased risk for HPgV-1 infection. Individuals who were single or without a fixed partner were twice as likely to be infected with the virus compared to those who were married or had a partner. Furthermore, while it was not a risk factor, the prevalence of HPgV-1 was found to be proportional to the number of sexual partners of

individuals per year, going from 8.3% in donors with no sexual partners to 33.3% among donors with ≥ 4 partners. Our study did not include persons with more than two sexual partners per year, since this is an exclusion criterion for blood donors during triage. Despite this, some reported to interviewers more than two partners per year. In face of this, we think the number of individuals with several sexual partners may have been underestimated, this being another limitation of this study.

In a study on the co-infection of HPgV-1 and HIV-1 in southern Brazil, having 1-3 sexual partners per year was considered a risk factor for HPgV-1 infection[26]. Sexual transmission, besides being quite efficient, is largely responsible for the spread of HPgV-1 in individuals who are not at risk of parenteral transmission[26,28,29]. This has been evidenced by the current study.

The present study represents the first time that circulating HPgV-1 strains have been identified in the southernmost region of Brazil. After phylogenetic analysis, genotype subtype 2b, showed the highest prevalence, followed by subtype 2a and, in smaller numbers, genotype 1. These findings suggest that the epidemiology of HPgV-1 in the southernmost region of Brazil is similar to that in the other regions of the country, with the exception of genotype 3, which has not yet been identified in this region. In the central and southeastern regions of Brazil, genotypes 2a and 2b represent the majority, while genotypes 1 and 3 are found to a lesser extent[10,12,14,22]. To confirm these findings, similar studies should be carried out in different cities in this region, since it has borders with other

countries, as well as ports that receive people from many diverse regions of the world, all of which could introduce different genotypes to the area.

The high similarity between some HPgV-1 sequences in the phylogenetic analysis (Figure 1) is noteworthy. Since we took multiple measures to avoid PCR contamination between samples, alternative explanations for that observation are raised. One would be the nature of the sequence region selected for HPgV-1 amplification, the 5' NCR. This is a highly conserved region with few genomic variations[30]. In addition, the amplified fragment was relatively short (344 bp), which further diminishes the number of phylogenetic informative sites that distinguish the amplified sequences. The epidemiological profile of donors may also have influenced the similarity between some sequences, since most donation candidates were members of the same family, such as spouses, children and other relatives for the reasons mentioned above in this section.

In conclusion, our data suggest a high endemicity of HPgV-1 infection in blood donors in southernmost Brazil. The co-circulation of genotypes 1 and 2 in this region, with a higher prevalence of HPgV-1 subtype 2b, was also demonstrated for the first time in this region. The similarities between some HPgV-1 sequences evidenced an important contribution of autochthonous transmissions in the area, and HPgV-1 was likely sexually transmitted in the individuals that were studied. Our data emphasizes the need to evaluate donor blood components as well as the individuals who receive blood transfusion for this virus.

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REFERENCES

1. Deinhardt F, Holmes AW, Capps RB, Popper H. Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. *J Exp Med.* 1967;125:673-688.

2. Chivero ET, Stapleton JT. Tropism of human pegivirus (formerly known as GB virus C/hepatitis G virus) and host immunomodulation: insights into a highly successful viral infection. *J Gen Virol.* 2015;96:1521-1532.

3. Adams MJ, King AM, Carstens EB. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2013). *Arch Virol.* 2013;158:2023-2030.

4. Stapleton JT, Chaloner K, Martenson JA, et al. GB virus C infection is associated with altered lymphocyte subset distribution and reduced T cell activation and proliferation in HIV-infected individuals. *PLoS One.* 2012;7:e50563.

5. Pavesi A. Origin and evolution of GBV-C/hepatitis G virus and relationships with ancient human migrations. *J Mol Evol.* 2001;53:104-113.

6. Berzsenyi MD, Roberts SK. What is the role of GB virus C infection in hepatitis C virus/HIV coinfection? *J Infect Dis.* 2006;194:407-409.

7. Feng Y, Zhao W, Feng Y, et al. A novel genotype of GB virus C: its identification and predominance among injecting drug users in Yunnan, China. *PLoS One.* 2011;6:e21151.

8. Fan X, Xu Y, Detre K, Di Bisceglie AM. Direct evidence for GB virus C/hepatitis G virus (GBV-C/HGV) superinfection: elimination of resident viral strain by donor strain in a patient undergoing liver transplantation. *J Med Virol.* 2002;68:76-81.

9. Wu H, Padhi A, Xu J, Gong X, Tien P. Evidence for Within-Host Genetic Recombination among the Human Pegiviral Strains in HIV Infected Subjects. *PLoS One.* 2016;11:e0161880.

10. Oliveira LA, Martins RM, Carneiro MA, et al. Prevalence and genotypes of GB virus C/hepatitis G virus among blood donors in Central Brazil. *Mem Inst Oswaldo Cruz.* 2002;97:953-957.

11. Nishiya AS, Ribeiro-dos-Santos G, Bassit L, Focaccia R, Chamone DF, Sabino EC. Genotype distribution of the GB virus C in citizens of Sao Paulo City, Brazil. *Rev Inst Med Trop Sao Paulo.* 2003;45:213-216.

12. Alcalde R, Nishiya A, Casseb J, Inocencio L, Fonseca LA, Duarte AJ. Prevalence and distribution of the GBV-C/HGV among HIV-1-infected patients under anti-retroviral therapy. *Virus Res.* 2010;151:148-152.

13. Campos AF, Tengan FM, Silva SA, Levi JE. Influence of hepatitis G virus (GB virus C) on the prognosis of HIV-infected women. *Int J STD AIDS.* 2011;22:209-213.

14. Giret MT, Miraglia JL, Sucupira MC, et al. Prevalence, incidence density, and genotype distribution of GB virus C infection in a cohort of recently HIV-1-infected subjects in Sao Paulo, Brazil. *PLoS One.* 2011;6:e18407.

15. Alves-Sousa VK, Komninakis SC, Baggio-Zappia GL, et al. Short communication: Evaluation of GB virus C/hepatitis G viral load among HIV type 1-coinfected patients in Sao Paulo, Brazil. *AIDS Res Hum Retroviruses.* 2012;28:1301-1304.

16. Feucht HH, Zollner B, Polywka S, et al. Prevalence of hepatitis G viremia among healthy subjects, individuals with liver disease, and persons at risk for parenteral transmission. *J Clin Microbiol.* 1997;35:767-768.

17. Abe K. GB virus-C/hepatitis G virus. *Jpn J Infect Dis.* 2001;54:55-63.

18. Ramia S, Mokhbat J, Sibai A, Klayme S, Naman R, Group HIVERS. Exposure rates to hepatitis C and G virus infections among HIV-infected patients: evidence of efficient transmission of HGV by the sexual route. *Int J STD AIDS.* 2004;15:463-466.

19. Santos LM, Lobato RC, Barral MFM, Goncalves CV, da Hora VP, Martinez AMB. Prevalence and vertical transmission of human pegivirus among pregnant women infected with HIV. *Int J Gynaecol Obstet.* 2017;138:113-118.

20. Mohr EL, Stapleton JT. GB virus type C interactions with HIV: the role of envelope glycoproteins. *J Viral Hepat.* 2009;16:757-768.

21. Ribeiro-dos-Santos G, Nishiya AS, Nascimento CM, et al. Prevalence of GB virus C (hepatitis G virus) and risk factors for infection in Sao Paulo, Brazil. *Eur J Clin Microbiol Infect Dis.* 2002;21:438-443.

22. Levi JE, Contri DG, Lima LP, et al. High prevalence of GB virus C/hepatitis G virus RNA among Brazilian blood donors. *Rev Inst Med Trop Sao Paulo.* 2003;45:75-78.

23. Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P. Infection with hepatitis G virus among recipients of plasma products. *Lancet.* 1996;348:1352-1355.

24. Hall TA. BioEdit: a user-friendly biological sequence alignmente ditor and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser.* 1999;41:95-98.

25. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 2011;28:2731-2739.

26. Da Mota LD, Nishiya AS, Finger-Jardim F, et al. Prevalence of human pegivirus (HPgV) infection in patients carrying HIV-1C or non-C in southern Brazil. *J Med Virol.* 2016;88:2106-2114.

27. Barbosa RM, Koyama MA, Grupo de Estudos em Populacao SeA. [Sexual behavior and practices among men and women, Brazil 1998 and 2005]. *Rev Saude Publica.* 2008;42:21-33.

28. Ramezani A, Mohraz M, Vahabpour R, et al. Frequency of hepatitis G virus infection among HIV positive subjects with parenteral and sexual exposure. *J Gastrointestin Liver Dis.* 2008;17:269-272.

29. Xiang J, Wunschmann S, Diekema DJ, et al. Effect of coinfection with GB virus C on survival among patients with HIV infection. *N Engl J Med.* 2001;345:707-714.

30. Appel N, Schaller T, Penin F, Bartenschlager R. From structure to function: new insights into hepatitis C virus RNA replication. *J Biol Chem.* 2006;281:9833-9836.

FIGURE

Figure 1. Neighbor-joining phylogenetic tree based on consensus sequences obtained from the 5'-NCR region of HPgV-1. Query sequences were aligned with reference sequences of HPgV-1 genotypes 1 through 7. Relevant *bootstrap* values (above 75%) are shown. Sequences generated in this study are marked with a green circle, while reference sequences are named after their GenBank accession number followed by the HPgV-1 genotype to which they are assigned. The scale bar under the tree indicates 0.01 nucleotide substitutions per site.

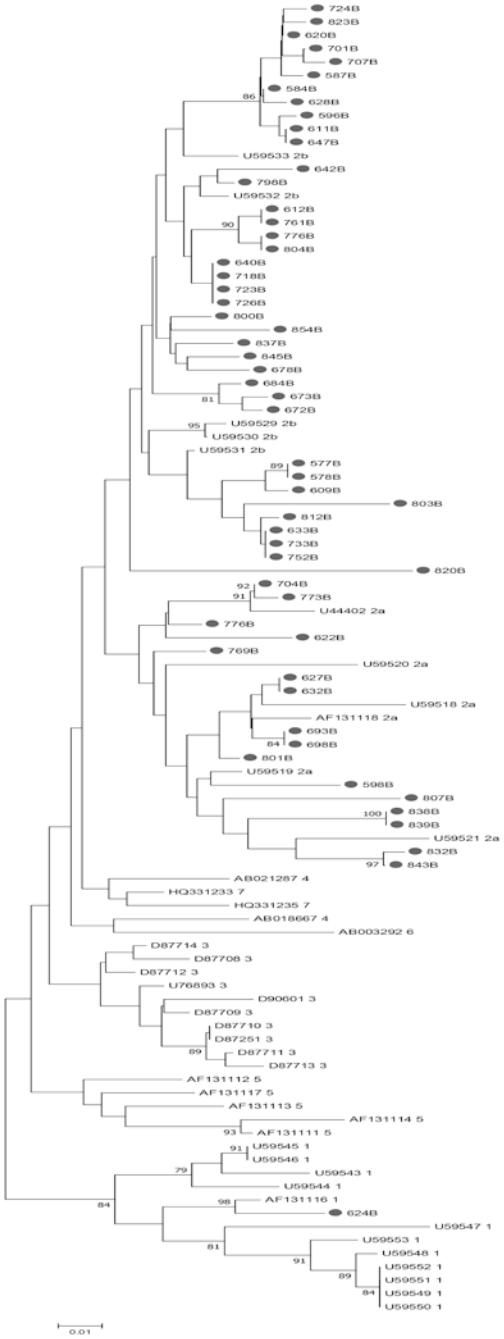

TABLE I: Sociodemographic and behavioral profiles of blood donnors analyzed in the study, stratified by HPgV-1 positivity

Variable/Category	n (%)	HPgV-1^{+}	Prevalence Ratio	95%CI	*P*[a]
Age					
≥ 51 years	31 (11)	4 (6.6)	1.0		
18-50 years	250 (89)	57 (93.4)	1.76	0.69-4.53	0.20
Ethnicity					
Non-white	51 (18.1)	6 (9.8)	1.0		
White	230 (81.9)	55 (90.2)	2.03	0.92-4.46	**0.05**
Gender					
Female	70 (24.9)	16 (26.2)	1.0		0.79
Male	211 (75.1)	45 (73.8)	1.07	0.65-1.77	
Education					
≤ 4 years	16 (5.7)	4 (6.6)	1.0		
5-8 years	59 (21)	12 (19.7)	0.81	0.30-2.18	0.91
≥ 9 years	206 (73.3)	45 (73.8)	0.87	0.36-2.12	
Relationship status					
Married / fixed partner	92 (32.7)	12 (19.7)	1.0		**0.01**
Single / no fixed partner	189 (67.3)	49	1.99	1.11-	
Income[b]					
<1Minimum wage (MW)	67 (23.8)	16 (26.2)	1.0		0.61
1-2 MW	93 (33.1)	17	0.76	0.41-	
> 2 MW	121 (43.1)	28	0.96	0.57-	
Residence					
Rural	19 (6.8)	7 (11.5)	1.0		0.09
Urban	262 (93.2)	54 (88.5)	0.56	0.30-1.05	
User of inhaled drugs					
No	251 (89.3)	56	1.0		0.74
Yes	30 (10.7)	5 (8.2)	0.74	0.32-	
Shared syringes or needles					
No	218 (77.6)	49 (80.3)	1.0		0.56
Yes	63 (22.4)	12 (19.7)	0.84	0.48-1.49	

Tattoo					
No	169 (60.1)	30 (49.2)	1.0		**0.04**
Yes	112 (39.9)	31	1.56	1.00-	
Number of sexual partners per					
None	12 (4.3)	1 (1.6)	1.0		0.25
1-3 partners	251 (89.3)	54 (88.5)	2.58	0.39-17.11	
≥4 partners	18 (6.4)	6 (9.8)	4.00	0.58-	
Already lived in another state or country					
No	167 (59.4)	33 (54.1)	1.0		0.33
Yes	114 (40.6)	28 (45.9)	1.24	0.79-1.98	

[a]Chi-square test

[b]MW at the time of the study of approximately US$ 300.00

Statistically significant results are indicated in bold.

TABLE II. Multivariate analysis of characteristics associated with HPgV-1 infection in a sample of blood donors of southern Brazil

Variables	Prevalence Ratio	95%CI	*P**
Age			
≥ 51 years	1.0		
18-50 years	0.63	0.20-1.97	0.43
Ethnicity			
Non-white	1.0		
White	0.44	0.17-1.10	0.08
Relationship status			
Married / fixed partner	1.0		
Single / no fixed partner	2.26	1.12-4.56	**0.02**
Residence			
Rural	1.0		
Urban	1.73	0.74-5.59	0.16
Tattoo			
No	1.0		
yes	1.71	0.96-3.05	0.07
Number of sexual partners per year			

None	1.0		
1-3 partners	3.10	0.38-25.00	0.28
≥ 4 partners	4.57	0.46-45.07	0.19

******P*-values in bold are statistically significant.**