Human pegivirus (HPgV, GBV-C) RNA prevalence, genome characterization and association with HIV coinfection among volunteer blood donors from a public hemotherapy service in Northern Brazil.

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Abstract

Background

Human pegivirus (HPgV) - formerly known as GBV-C - is a member of the Flaviviridae family and belongs to the species Pegivirus C. It is a non-pathogenic virus and is transmitted among humans mainly through the exposure to contaminated blood and is often associated with human immunodeficiency virus (HIV) infection. This study aimed to determine the prevalence of HPgV viremia, its association with HIV and clinical epidemiological factors, as well as the full-length sequencing and genome characterization of HPgV recovered from blood donors of the HEMOPA Foundation in Belém-PA-Brazil.

Methods

Plasma samples were obtained from 459 donors, tested for the presence of HPgV RNA by the RT-qPCR. From these, a total of 26 RT-qPCR positive samples were submitted to the NGS sequencing approach in order to obtain the full genome. Genome characterization and phylogenetic analysis were conducted.

Results

The prevalence of HPgV was 12.42%. We observed the highest prevalences among donors aged between 18 to 30 years old (16.5%), with brown skin color (13.2%) and men (15.8%). The newly diagnosed HIV-1 prevalence was 26.67%. The genotype 2 (2A and 2B) was identified. No data on viral load value was found to corroborate the protective effect of HPgV on HIV evolution.

Conclusions

This study provided information regarding the HPgV infection among blood donors from HEMOPA Foundation. Furthermore, we genetically characterized the HPgV circulating strains and described by the first time the genotype 2 genomes in the Brazilian Amazon region.

Background

Human pegivirus (HPgV), formerly known as GBV-C or hepatitis G virus (HGV), is a member of the Flaviviridae family, belongs to the species Pegivirus C [1]. HPgV is an enveloped virus with a single-stranded, positively polarized RNA genome comprising approximately 9,400 nucleotides. The viral
genome is similar to the genome of the hepatitis C virus and contains a single open reading frame (ORF) located between the untranslated regions (UTRs) at the 5¢ and 3¢ ends of the viral genome. The 5¢-NTR region is highly conserved with an internal ribosome entry site (IRES) and is responsible for the initiation of the translation of the viral RNA, resulting in the synthesis of a polyprotein of approximately 3,000 amino acid residues. Through the action of cellular peptidases and viral proteases, the polyprotein is cleaved to produce eight mature yet incompletely characterized proteins, including the two structural (E1 and E2) and seven non-structural (NS) proteins [2–4].

HPgV is transmitted among humans mainly through exposure to contaminated blood. This transmission profile deems HPgV as a common coinfection with other viruses such as HIV-1, hepatitis C virus (HCV), and Ebola virus [5–7]. Up to 40% of the individuals infected with HIV and/or HCV are positive for HPgV infection [8,9]

People HIV-1 co-infected with HPgV experience slower disease progression that may be influenced by the interference of HPgV on the pathogenicity of HIV-1, however, the mechanism by which HPgV mediates this protective effect still remains unknown [10,11].

Several studies carried out in different populations in the last decades in Brazil have shown varying prevalence rates [12,13]. In studies among healthy blood donors conducted in Brazil, prevalence rates of 19.5% and 9.7% were observed among individuals with prior exposure and active infection, respectively [14]. However, the most significant prevalence has been reported among patients with HIV, with a value reaching up to 34% [15]. The prevalence of the virus is lower in the developed countries (1-5%) than in the developing countries -(approximately 20%), with South America exhibiting a prevalence rate of up to 14.6% among blood donors [4]. Seroprevalence studies in Brazil reveal the presence of anti-E2 antibodies in 19.5% of healthy blood donors [16], however, little is known about the prevalence of HPgV viremia and its circulating strains among the Brazilian population, particularly blood donors.

This study aimed to determine the prevalence of HPgV viremia, its association with HIV and clinical epidemiological factors, as well as the complete genome characterization of HPgV present in blood donors of the HEMOPA Foundation in Belém-PA-Brazil.
Methods
Blood donors and the collection of serum samples
A cross-sectional study was performed in order to determine the prevalence of HPgV infection among blood donors from the HEMOPA Foundation between March 2017 and April 2018. Epidemiological data were obtained through access to the HEMOPA Foundation donor registry. The sample size was calculated using EpilInfo™ software [17] based on the presumed prevalence of 5-10% of HPgV in Brazil [15, 18]. For this calculation, the number of blood donors registered in 2016 at the HEMOPA Foundation (63,501), 95% confidence level, and 20% margin adjustment was used to obtain a total of 366 individuals. A total of 459 serum samples (400 µL) from the blood donors from the HEMOPA Foundation were tested.

Extraction and detection of HIV, HCV, and HPgV nucleic acids
The extraction of nucleic acids was performed using the QIAmp RNA mini Kit (Qiagen®, Hilden, Germany) according to the manufacturer’s recommendations. HIV and HCV detection were performed with Hemocenter’s Nuclear Acid Test Platform (NAT) using the HIV/HCV NAT kit (Bio Manguinhos®, Rio de Janeiro, Brazil), according to the manufacturer’s recommendations.

The presence of HPgV nucleic acid was evaluated by the RT-qPCR, using the custom Assay TaqMan® Fast Virus 1-Step, developed by AB Applied Biosystems (Foster City, California, EUA), following the manufacturer’s Fast protocol as follows: 1 cycle of reverse transcription (RT) for 2 min at 50 °C; inactivation of Reverse Transcription (RT)/start of denaturation (1 cycle) for 20 s at 95°C; amplification for 40 cycles of 95 °C for 3 s and 60 °C for 30 s. The selected primers corresponded to the 5′-UTR of the viral genome according to GenBank NC_001710 and were as follows: RTG1 (GTGGTGGATGGGTGATGACA; sense), RTG2 (GACCCACCTATAGTGGCTACCA; antisense), and NFQ (5′-FAM-CCGGGATTACGACCTACC3′; probe) [15].

Quantification of HIV-1 and HPgV plasma viral load
HIV-1 viral load was measured in a Real-Time Rotor-Gene® Q platform using artus HI Virus-1 RG RT-PCR (QIAGEN Hilden, Germany) and HPgV viral load was measured in a Real-Time LightCycler® 480 Instrument II (Roche Applied Science, Penzberg, Germany) using TaqMan® Fast Virus 1-Step Master Mix (Foster City, California, USA). Both methods strictly followed the manufacturer’s recommendation.

Sequencing
The RNA, obtained in the nucleic acid extraction step, was quantified in Qubit 2.0 fluorometer (Thermo Fisher Scientific), using the QubitTM RNA HS Assay Kit 500 assays (Invitrogen by Thermo Fisher Scientific). Then, cDNA was synthesized using the cDNA Synthesis System Roche® kit (Roche Applied Science), as described by the manufacturer. The subsequent step was the quantification of cDNA using the qubit 2.0 fluorometer (Thermo Fisher Scientific), using the QubitTM dsDNA HS Assay Kit (Invitrogen by Thermo Fisher Scientific) and analysis of cDNA integrity in the equipment 2100 Bioanalyzer (Agilent Technologies) using the high sensitivity DNA reagents kit (Agilent Technologies). Genome sequencing was performed using the HiSeq 2500 platform (Illumina) as previously described [19].

Bioinformatics Analysis
Generated reads were filtered, adapters and reads with Phred quality scores below 20 and size less than 50 nt, were removed using Trim Galore 0.4.4, Cutadapt and Prinseq-lite 0.20.4 software [20–22]. The filtered reads were used in de novo assembly strategy applying two software: IDBA-UD v.1.1.3 [23] and MEGAHIT v.1.1.3 [24], both set to a k-mer range of 21 to 91, varying every 10 k-mer. For the removal of redundant data, generated contigs were processed using CD-Hit-Est v.4.7 [25] set to a threshold of 90% identity. Then, the non-redundant contigs were aligned against the NCBI non-redundant protein database using the Blastx (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) algorithm and the software Diamond v.0.9.22 [26].

Genome characterization and phylogenetic inference
The HPgV genomes, identified by the Blastx algorithm, were used for predicting the coding region (Open Reading Frame; ORF), as well as the 5'-UTR and 3'-UTR regions using the Geneious v9 tool. Viral genomes were aligned with other HPgV complete genomes available in GenBank database using the MAFFT v7 software [27]. For phylogenetic analyses, ORFs from aligned genomes were used to perform the phylogenetic reconstructions using the maximum-likelihood method, generated by RAxML v.8.2.12 [28], applying 1000 bootstrap replicates[29] and the best nucleotide replacement model calculated by JModelTest [30]. Complete genome sequences of HPgV obtained in this study have been
submitted to GenBank (accession numbers MN215894–MN21591).

**Statistical analysis**

The differences between the groups were analyzed with the chi-square test, G test of independence, Student’s t-test, and odds ratio. The level of significance of $\alpha = 0.05$ was adopted for the rejection of the null hypothesis. Statistical analyses were performed using the BioEstat program version 5.3 and GraphPad Prism version 8; Microsoft Excel Professional 2007 program was used for data processing and to prepare tables and databases.

**Results**

Plasma samples were obtained from 459 donors at the time of screening. The prevalence of HPgV in the samples was 12.42% ($n = 57$) and a total of 26.14% ($n = 120$) of the donors were HIV positive, diagnosed through routine tests carried out at the HEMOPA Foundation. The HPgV prevalence among HIV donors was 26.67% ($n = 32$, $p < 0.0001$). HIV positive donors were four times more likely to have HPgV infection than those HIV negative (odds ratio = 4.56, $p < 0.0001$, 95% confidence interval [CI]:2.57–8.10).

The highest prevalences of HPgV were observed among donors with ages ranging from 18 to 30 years old (16.5%, $p = 0.024$), men (15.8%, $p = 0.005$) with brown skin color (13.2%, $p = 0.462$) and 12 or more years of education (24.0%, $p < 0.0001$) (Table 1).
Clinical epidemiological data on the prevalence of HPgV among blood donors.

| Variable/category | Total | HPgV+ | HPgV− | p-value |
|-------------------|-------|-------|-------|---------|
|                   | n     | (%)   | n     | (%)     | n     | (%)   |
| **Age (years)**   |       |       |       |         |       |       |
| 18–30             | 224   | 48.8% | 37    | 16.5%   | 187   | 83.5% |
| p = 0.0273        |       |       |       |         |       |       |
| 31–50             | 197   | 42.9% | 18    | 9.1%    | 179   | 90.9% |
| ≥ 51              | 38    | 8.3%  | 2     | 5.3%    | 36    | 94.7% |
| **Skin color**    |       |       |       |         |       |       |
| Brown             | 356   | 77.6% | 47    | 13.2%   | 309   | 86.8% |
| White             | 84    | 18.3% | 9     | 10.7%   | 75    | 89.3% |
| Black             | 19    | 4.1%  | 1     | 5.3%    | 18    | 94.7% |
| **Sex**           |       |       |       |         |       |       |
| Male              | 284   | 61.9% | 45    | 15.8%   | 241   | 84.9% |
| Female            | 175   | 38.1% | 12    | 6.9%    | 161   | 92.0% |
| **Education (years of study)** |       |       |       |         |       |       |
| ≥ 12              | 146   | 31.8% | 35    | 24.0%   | 111   | 76.0% |
| 9 to 11           | 259   | 56.4% | 19    | 7.3%    | 240   | 92.7% |
| ≤ 8               | 54    | 11.8% | 3     | 5.6%    | 51    | 94.4% |
| **Co-infection**  |       |       |       |         |       |       |
| HIV+              | 120   | 26.1% | 32    | 26.7%   | 88    | 73.3% |
| HIV−              | 339   | 73.9% | 25    | 7.4%    | 314   | 92.6% |

In 18 of the 57 HPgV positive samples (31.6%) near-complete genomes sequences were obtained. The BlastX result of the 18 genomes obtained showed 91–93.24% identity with Human pegivirus sequences from the United Kingdom (LT009489 and LT009494), France (MH053115) and Japan (D87255), available from GenBank / NCBI (Table 2). The pairwise alignment of the polyprotein amino acid sequences of these four strains of the bank along with the 18 described sequences showed an identity of 98.6%.

**Table 2**

| Sample | Genome Length | Mean Cover | Best Hit | Query cover | E-value | Identity | Accession |
|--------|---------------|------------|----------|-------------|---------|----------|-----------|
| P01    | 8,392         | 14,8       | Human pegivirus isolate 56330229 | 100% | 0 | 92.14% | LT009489 |
| P02    | 8,995         | 20,1       | Human pegivirus isolate D2B2C | 99% | 0 | 91.00% | MH053115 |
| P09    | 8,933         | 22,9       | Human pegivirus | 99% | 0 | 92.34% | LT009489 |
|   |   |   | isolate 56330229 | isolate 56330229 | 100% | 0 | MH053115 |
|---|---|---|---|---|---|---|---|
| P13 | 9,101 | 593,8 | Human pegivirus isolate D2B2C | Human pegivirus isolate D2B2C | 100% | 0 | MH053115 |
| P21 | 9,172 | 448,6 | Hepatitis G virus | Hepatitis G virus | 100% | 0 | D87255 |
| P22 | 8,811 | 26 | Human pegivirus isolate D2B2C | Human pegivirus isolate D2B2C | 99% | 0 | MH053115 |
| P23 | 9,190 | 133,7 | Human pegivirus isolate D2B2C | Human pegivirus isolate D2B2C | 99% | 0 | MH053115 |
| P24 | 9,306 | 386 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 99% | 0 | LT009489 |
| P25 | 9,241 | 327,1 | Human pegivirus isolate D2B2C | Human pegivirus isolate D2B2C | 99% | 0 | MH053115 |
| P26 | 9,189 | 143,7 | Human pegivirus isolate D2B2C | Human pegivirus isolate D2B2C | 99% | 0 | MH053115 |
| P27 | 8,873 | 22,6 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 99% | 0 | LT009489 |
| P28 | 8,913 | 32,5 | Hepatitis G virus | Hepatitis G virus | 99% | 0 | D87255 |
| P31 | 9,521 | 538,5 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 99% | 0 | LT009489 |
| P32 | 9,256 | 182,7 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 99% | 0 | LT009494 |
| P33 | 9,409 | 640 | Human pegivirus isolate 56330286 | Human pegivirus isolate 56330286 | 100% | 0 | LT009494 |
| P34 | 9,270 | 146,1 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 100% | 0 | LT009489 |
| P35 | 9,198 | 755,6 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 100% | 0 | LT009489 |
| P55 | 9,203 | 566,4 | Human pegivirus isolate 56330229 | Human pegivirus isolate 56330229 | 100% | 0 | LT009489 |

All nearly complete genome sequences showed the common genome organization related to members of the HPgV: unique and large ORF flanked by 5’and 3’ UTRs; two structural proteins (E1 and E2) and seven nonstructural proteins (p5,6, NS2, NS3, NS4A, NS4B, NS5A, NS5B). The phylogenetic tree depicted seven major groups (genotype1, 2a, 2b, 3, 4, 6 and 7) and two subgenotypes (2A and 2B) with high bootstrap values (> 90%). All generated sequences clustered with HPgV genotype 2 sequences (Fig. 1).
We found a higher viral load \((2.72 \text{ Log}_{10})\) of HIV-1 in the confected group (HIV + HPgV positive, Fig. 2a), while a higher viral load \((4.28 \text{ Log}_{10})\) of HPgV was found in the monoinfected group (HPgV positive, Fig. 2b).

**Discussion**

The prevalence of HPgV-1 among blood donors was 12.4%, which is consistent and not significantly different from the expected prevalence in developing countries (up to 20%) [4, 31]. The prevalence calculated in this study was 2.8% higher than reported Slavov et al. [32] in a study among blood donors from the city of Macapá (northern Brazil). Previous studies have shown that the prevalence of HPgV among blood donors in most regions of Brazil varies from 5–10% [14, 33–36], although Da Mota et al. [37] have found a high prevalence of 21.7% in the southernmost region of Brazil.

A significant association was observed between the presence of HPgV and age, gender, and years of education by Miranda et al. [38]. In our findings, the highest prevalence of HPgV occurred among subjects between 18 and 30 years of age (16.5%), males (15.8%), and brown individuals (13.2%). It is important to highlight that the epidemiological profile of the donors was similar to that observed in the epidemiology of HIV/AIDS in Brazil, wherein the majority of the infected individuals were male and young subjects (15 to 39 years) with up to 11 years of age (completed high school) [39]. Another issue to consider is that sexual activity is more evident among young people so this population has shown a greater risk to be infected with HPgV, as suggested Da Mota et al. [15] and Miao et al [40]. The prevalence of HPgV among the individuals diagnosed with HIV-1 in this study was 9.7% higher than that reported by Miranda et al. [38]. The high prevalence of HPgV among HIV-1 individuals has been reported in several studies in Brazil and the world [40–42]. The association between the presence of HPgV and HIV is owing to the fact that HPgV likely acts as a protective factor for the development of HIV [40, 43, 44].

HIV-1 infected people have reduced mortality when co-infected with HPgV, nonetheless the mechanism by which HPgV mediates this protective effect remains unknown [45, 46]. Nevertheless, the present study showed no evidence of viral load value that corroborated with the protective effect of HPgV in the evolution of HIV, instead, HIV-1 viral load in the coinfected group (HIV-1 + HPgV
positive) was 0.72 $\log_{10}$ ($p = 0.002$) higher than in a monoinfected group (HIV-1 positive). One interpretation of this finding is that the increase in T cells during the expansion phase of viral infection leads to an increase in both viral loads [47, 48]. Another consideration is that all individuals in our sample were newly diagnosed with HIV during the acute phase, suggesting that HPgV does not exert a protective effect on the pathogenesis of HIV during the acute phase of HIV infection as suggested Bailey et al. [49]. We hypothesize that HIV-1 would have an advantage in lymphocyte infection since HPgV may infect the same cells as HIV-1 [50].

The phylogenetic analysis revealed the presence of genotype 2 and the subtypes 2a and 2b in the studied population. These findings corroborate previous studies that identified these same genotypes in other regions of Brazil [18, 33, 41] and in Brazilian Amazon [32].

HPgV is known as a non-pathogenic virus and is not part of the routine diagnosis in the HEMOPA Foundation, but further studies are necessary to evaluate the unclear aspects related to HPgV infection especially those related to viral biology and interaction with HIV-1. This study genetically characterized and identified, by the first time, the circulating strains of HPgV among blood donors from HEMOPA Foundation and described by the first nearly complete genomes of genotype 2 in Brazilian Amazon.

Conclusions
This study provided information regarding the HPgV infection among blood donors from HEMOPA Foundation. Furthermore, we genetically characterized the HPgV circulating strains and described by the first time the genotype 2 genomes in the Brazilian Amazon region.

List Of Abbreviations
cDNA - Complementary deoxyribonucleic acid
dsDNA - Doble-stranded deoxyribonucleic acid
GBV-C - GB virus C
HCV - Hepatitis C virus
HEMOPA Foundation - Foundation Center for Hemotherapy and Hematology of Pará
HIV - Human immunodeficiency virus
HPgV - Human Pegivirus

IRES - internal ribosome entry site

ORF - open reading frame

RNA - Ribonucleic acid

RT-qPCR- Reverse transcription real-time polimerase chain reaction

UTRs - Untranslated regions

Declarations

**Ethics approval and consent to participate**

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This work was approved by the Human Research Ethics Committee of the State University of Pará (Opinion No. 1.868.004/CAAE: 62348616.4.0000.5174) and all donors were informed of their participation in this research project by signing the Term Free and Informed Consent (EHIC) and all the privacy rights were observed.

**Consent for publication**

Not applicable

**Availability of data and materials**

Not applicable

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

AS contributed to the conception of the work; acquisition, analysis and interpretation of data for the work; revising the work critically for important intellectual content. CS worked on the acquisition,
analysis and interpretation of data RB contributed to the acquisition, analysis and interpretation of the data. PS and PM contributed on the acquisition of data. LL and RB contributed to the conception of the work; acquisition, analysis and interpretation of data for the work; revising it critically for important intellectual content. MN and PM contributed to the conception of the work; acquisition, analysis and interpretation of data for the work; revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Figures

Figure 1

Phylogenetic tree of Human pegivirus (HPgV) generated using RAxML with the GTR+I+G+F nucleotide substitution model using 1000 bootstrap replicas displaying only values greater than 50.
Virological profiles of newly diagnosed blood donors with HIV-1 and HPgV. (A) Comparison of plasma HIV-1 viral load between HIV-1 monoinfected group and HIV-1/HPgV coinfected group. (B) Comparison of plasma HPgV viral load between HPgV monoinfected group and HPgV/HIV-1 coinfected group.