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Short report

Molecular identification of the emerging Human Gemykibivirus-2 (HuGkV-2) among Brazilian blood donors

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ABSTRACT

Human gemykibivirus-2 (HuGkV-2) belonging to the Gemykibivirus genus (Genomoviridae family) is an emerging DNA virus which has been described as a component of the virome of a wide variety of samples including clinical ones. So far, the HuGkV-2 DNA prevalence in the human population as well as its clinical impact are completely unknown. The objective of this study was to investigate the HuGkV-2 DNA prevalence among Brazilian healthy blood donors from three different geographic regions. A total of 450 blood samples were screened for HuGkV-2 DNA (150 samples were from the Brazilian Amazon, 150 from Midwest Brazil and 150 from South Brazil). The overall HuGkV-2 DNA prevalence was 7.8 %. Considering the examined regions, the highest prevalence was observed in the Brazilian Amazon (city of Macapa, state of Amapa), 15.3 %, followed by the Midwest Brazil (city of Brasilia, Federal District) (6.0 %) and South Brazil (city of Santa Maria, Rio Grande do Sul State) (2.0 %). This study gives preliminary insights on the molecular prevalence of HuGkV-2 DNA among Brazilian blood donors, highlighting that the highest HuGkV-2 prevalence was recorded in the Brazilian Amazon. However, more studies regarding the prevalence, transmission routes and any possible clinical effects appear to be crucial in order to understand the impact of this emerging viral agent.

1. Introduction

Human gemykibivirus 2 (HuGkV-2) belongs to the *Gemykibivirus* genus of the *Genomoviridae* family. Genomoviruses include small (~2.4 kB), circular and single-stranded DNA viruses. The identification of these viruses has been possible due to the wide application of next-generation sequencing and metagenomic approaches on different types of environmental, plant, animal and human samples [1]. Studies report a wide host range of the gemikibiviruses including insects [2–4], birds [5–7] and humans [8,9]. Gemykibiviruses have also been isolated from sewage waters [10].

The first evidence that HuGkV-2 might be involved in human infection was reported in 2019, when applying viral metagenomics in a Chinese patient with unexplained acute respiratory syndrome a complete HuGkV-2 genome was recovered [8]. Further, also applying metagenomics, HuGkV-2 genomic sequences have been identified in plasma

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samples from Brazilian blood donors from the Amazon who were positive for HIV and HBV [9,11]. In all these studies the presence of HuGkV-2 DNA has been directly confirmed by molecular methods.

The presence of HuGkV-2 blood phase naturally raises questions related to viral transmission by blood transfusion and HuGkV-2 prevalence in blood donors. Although currently, we have no relationship between HuGkV-2 presence and clinical effect, there is growing evidence that a closely related group of viruses, the gemycircularviruses, have been involved as possible causes for encephalitis, respiratory diseases, sepsis, pericarditis, diarrhea and multiple sclerosis [12–18]. Therefore, more detailed studies are needed in order to examine the clinical impact of HuGkV-2. Additionally, great majority of the transfused patients show varying levels of immune suppression which further emphasizes that even non-harmful e commensal viruses for the healthy population might demonstrate different clinical behavior in patients with compromised immune functions.

Therefore, the objective of this study was to examine the prevalence of HuGkV-2 DNA in blood samples obtained from eligible blood donors from three different Brazilian regions i.e., North, Midwest and South Brazil. We selected these three different Brazilian regions to obtain a more complete image of the HuGkV-2 prevalence and its variation nationwide.

2. Materials and methods

2.1. Clinical samples

A total of 450 serum samples from blood donors were screened for HuGkV-2. Between January-May, 2015, 150 blood samples were obtained from the North region (city of Macapá, Amapá state). For the period between July-August, 2019, 150 blood samples were obtained from Midwest Brazil (city of Brasilia, Federal District). Finally, between December, 2016 -January, 2017, 150 blood samples were also obtained from the South region (city of Santa Maria, Rio Grande do Sul state) (Fig. 1A). Ethical permission for realization of this study was obtained from the Institutional Ethics Committees of the Faculty of Medicine of Ribeirao Preto, University of São Paulo (CAAE 05905719.5.0000.5440), Franciscan University of Santa Maria (Project number 3.279.167) and the Health Secretariat of the Federal District of Brazil (protocol number 62718016.0.3001.5440). The samples were initially centrifuged for sedimentation of the cellular component and were further stored at − 80 °C util use.

2.2. Nucleic acids extraction and HuGkV-2 DNA amplification

Nucleic acids were manually extracted from 140 µl of serum a using the QIAamp Viral RNA Mini Kit (Qiagen), following the manufacturer's instructions. HuGkV-2 DNA detection was performed using in-house optimized nested-PCR with primer sequences shown in Table 1. The primers were manually designed on an assembled HuGkV-2 contig of ~ 300 bp obtained from a blood donor from the Brazilian Amazon during the metagenomic analysis. The approximate positions of the primers towards other HuGkV-2 genomes available in the GenBank as well as their characteristics are showed in Table 1.

The amplification was performed using 1X PCR Buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 1.25 U of Platinum Taq DNA Polymerase (ThermoFisher Scientific), 400 nM of each primer and 5 µl of extracted DNA in a 50 µl final volume reaction mix. The cycling conditions of the first reaction included an initial denaturation at 95ºC for 5 min, followed by 37 cycles of 95 ºC for 30 s, 55 ºC for 30 s and 72ºC for 1 min, and a final extension 72ºC for 10 min. The amplification was performed in a Veriti 96-well Thermal Cycler (ThermoFisher Scientific). For the second reaction, we used 2 µl of the amplification product obtained in the first reaction and the same concentrations of reagents. The amplification conditions were the same as the first reaction with the difference that 40 cycles were applied. The visualization of the amplicons was carried out in 2 % agarose gel in ChemiDoc XRS+ with Image Lab Software (Bio-Rad). The positive samples for HuGkV-2 DNA were double confirmed in order to reproduce the positive results. All of the steps were performed in separate laboratory rooms to prevent contamination.

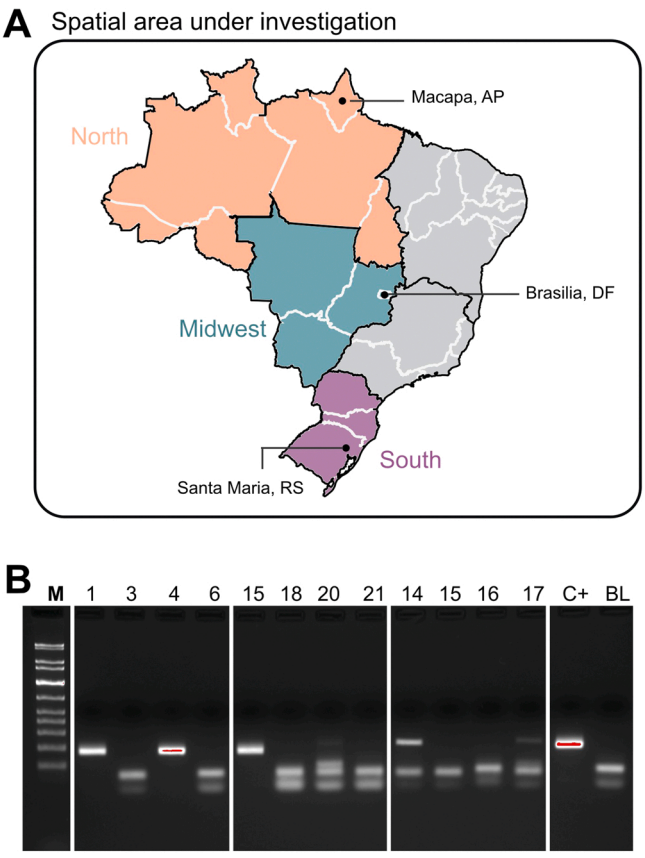

Fig. 1. Spatial area of investigation and molecular detection of Human Gemykibivirus-2 (HuGkV-2) in Brazilian blood donors from different regions. A) Map of Brazil showing the regions from where blood samples were obtained from eligible donors: i) North (Macapá city, Amapá state); ii) Midwest (Brasilia city, Federal District); iii) and South (Santa Maria city, Rio Grande do Sul state). The color indicates different Brazilian regions. B) Gel electrophoresis of amplification reaction of HuGkV-2 for samples obtained from different Brazilian regions. From left to right: M, molecular marker (100 bp plus Ladder, GelPilot DNA molecular Weight, QIAGEN); 1–6, blood donor samples from the Macapa city, samples 1 and 4 show the appropriate HuGkV-2 band with approximate size ~ 183 bp; 15–21 blood donor samples obtained from the Rio Grande do Sul State (city of Santa Maria), sample 15 shows positive amplification with the appropriate band size; 14–17 blood donor samples obtained from the Federal District of Brazil, sample 14 shows positive amplification and 17 weak amplification. C+ : Positive amplification control; BL: blank amplification reaction.

2.3. Statistical analysis

The online tool (www.winepi.net) [19] was used to calculate the prevalence of HuGkV-2 DNA found in our study and the estimated true prevalence considering the total number of blood donations obtained for the studied period from each location. The standard deviation was calculated using GraphPad v.5.

3. Results

3.1. Demographics of the tested blood donors in each location

The blood donors from the Brazilian Amazon (city of Macapa) showed a mean age of 35.62 years of age (range 19–56 years of age),

Table 1
Characteristics of the used nested-PCR primers for detection of Human Gemykibivirus-2 (HuGkV-2).

Reaction	Primer designation	Position	Reference genomes	Sequence (5′–3′)	Product length (bp)
First round PCR	GKV-F	1773–1793	MH734235 MN765188 MK513443	CGTGGATCATGTGCCTATAAG ————————————————————————————ATAAGC	236 bp
	GKV-R	1990–2009		CAGATCTCGTCGTACCAGCA —C————————————————————————————	
Second round PCR	GKVN-F	1804–1823		AACTCTTCTCGACTTGTTGCA	182 bp
	GKVN-R	1965–1986		TTGATGTCGACAATCATCACCC	

standard deviation (SD)= 9.15, margin of error (ME) 0.74 and the gender distribution was as follows: 107 males (71.3 %) and 43 females (28.7 %). From Midwest Brazil (the city of Brasilia), the mean age of the donors was 29.9 years of age (range 17–63 years of age), SD= 8.62, ME= 0.70 and the gender distribution was 66 (44.0 %) males and 84 females (84.0 %). Ultimately, from South Brazil (city of Santa Maria), the donors were comprised of 94 male (62.7 %) and 56 (37.3 %) female individuals who showed a mean age of 34.9 years of age (range 17–69 years of age), SD= 8.8, ME= 0.72.

3.2. Prevalence of HuGkV-2 DNA among blood donors

The overall prevalence of HuGkV-2 DNA among the blood donors was 7.78 % (n = 35/450). Among all tested regions the highest HuGkV-2 prevalence was observed in the Brazilian Amazon (city of Macapa): 15.33 % (n = 23/150). The estimated true prevalence for this region was between 9.7 % and 21.0 % (CI:95 %, based on total number of blood donations for the studied period was 4401). The second highest prevalence was observed in Midwest Brazil (city of Brasilia), (6.0 %, n = 9/150). The estimated true prevalence for this Brazilian region was between 2.2 % and 9.8 % (CI:95 %, based on total number of donations for the studied period: 8736). Finally, the lowest HuGkV-2 DNA prevalence was observed in South Brazil (2 % n = 3/150). The estimated true prevalence for the region was between 0.1 % and 3.9 % (CI:95 %, total number of collected samples for this period 501). Amplification results of the HuGkV-2 detection by region are shown in Fig. 1B.

4. Discussion

In this study, we estimated the prevalence of HuGkV-2 DNA among healthy Brazilian blood donors from three distinct regions. With the advent of the next-generation sequencing techniques and the identification of human gemykibiviruses in blood samples are raised concerns if the HuGkV-2 can be transmitted by blood transfusion. This is additionally supported by the fact that we have no data about HuGkV-2 clinical impact, transmission routes and prevalence in the human population.

The overall HuGkV-2 prevalence in Brazilian blood was 7.78 %. The highest HuGkV-2 DNA prevalence was observed in blood donors from the Brazilian Amazon. Previous metagenomic studies performed by our group, showed also the presence of HuGkV-2 reads in that region compared to other Brazilian locations, especially South Brazil [9,11]. We can hypothesize a local endemicity of HuGkV-2 in this region but due to the lack of larger studies that examine HuGkV-2 prevalence in the general population it is difficult to perform such an affirmation. In that line, an important limitation of our study is that we tested a selected group of individuals (blood donors) who cannot be compared to the general population. Therefore, we believe that the observed HuGkV-2 DNA prevalence in our study might be underestimated. To overcome this problem, studies including different age groups are necessary to more precisely estimate the HuGkV-2 dissemination in the general population.

The clinical impact of HuGkV-2 remains unknown. HuGkV-2 has been detected in the lower respiratory tract of a patient with severe respiratory distress in China [8] by viral metagenomics. As cited above, this virus has also been identified among blood donors positive for the routinely tested blood-borne infection [9,11] during next-generation sequencing studies. It is unknown if HuGkV-2 might be regarded as a coincidental finding or can be correlated to a clinical disease. However, phylogenetically a closely related group of viruses like the gemycircularviruses have been suggested as possible causes of a wide range of clinical symptoms [12–18] that suggests that HuGkV-2 might also show clinical significance. In that line, an important question is if routine testing for HuGkV-2 DNA in blood donors is also needed. We believe that as we do not have sufficient information for the transmission routes and clinical impact of HuGkV-2, at least at this moment, a routine testing for this viral agent is unfeasible. We also understand that it is impossible to routinely test all viruses which could represent a transfusion threat, but studies which evaluate and describe possible emerging threats are important in order to elaborate alternative strategies for improvement of the quality of transfused blood and attract our attention to viral pathogens that could represent transfusion threats in the near future.

In conclusion, the performed study gives preliminary insight on the molecular prevalence of the putative viral agent HuGkV-2 among Brazilian blood donors from three different regions. Due to the lack of information considering the clinical impact and transmission routes of this virus, we believe that currently no routine testing is warranted. We observed the highest prevalence of HuGkV-2 was observed in the Amazon region. However, more detailed studies are necessary to elucidate the dissemination, transmission routes and clinical impact of this emerging virus.

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CRediT authorship contribution statement

SNS: Conceptualization, SNS, MG, VSZ, DCCA, RMAM, BMSP, RH HTB: Data curation, SNS, MG, RH, MC, LCAJ, SK, DTC: Formal analysis, SNS: Funding acquisition, VZS, LK, DCCA, LOAC, RMAM, BMSP, RH, MC, LCAJ, SL and DTC: Investigation, VZS, SNS, MG: Methodology, SNS, RH, MG: Software, SNS; Supervision, SNS, MG and MC: Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interests.

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