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MAJOR ARTICLE

The Impact of Human Pegivirus on CD4 Cell Count in HIV-Positive Persons in Botswana

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Background. Human pegiviruses (HPgV)—formerly known as hepatitis G virus or GB virus C (GBV-C)—are common single-stranded RNA viruses that may have a beneficial impact on slowing HIV disease progression. The data on HPgV in resource-limited regions such as Sub-Saharan Africa are scarce. Thus, we conducted the first study of HPgV in Botswana as part of a natural history study of HIV subtype C disease progression.

Methods. Plasma samples from 133 HIV-positive adults were evaluated for HPgV RNA, and the 5'UTR was sequenced to determine the HPgV genotype.

Results. HPgV RNA was detected in 41 (30.8%) individuals. While the presence of HPgV RNA had no impact on baseline HIV viral load, a significant difference in baseline CD4 cell count was observed. HPgV genotypes were determined for 27 individuals and included 5 individuals (18.5%) with genotype 1 and 22 (81.5%) with genotype 5. Baseline CD4 cell counts were significantly higher for persons infected with HPgV genotype 5 compared with genotype 1.

Conclusions. These data suggest that HPgV infection is common among HIV-positive individuals in Botswana and has a significant impact on CD4 cell count. This difference in CD4 cell count based on HPgV genotype suggests that HPgV genotype should be evaluated as a possible predictor of HIV disease progression and highlights the need for additional studies of this virus in resource-limited settings.

Keywords. Africa; Botswana; hepatitis G virus; HIV; human pegivirus (HPgV); GB virus C (GBV-C); genotype.

The human pegivirus (HPgV)—originally described as hepatitis G virus or GB virus C (GBV-C)—is a positive-strand RNA virus that is distantly related to hepatitis C virus. HPgV has gained notoriety due to its possible beneficial impact on HIV disease progression. The prevalence of HPgV RNA ranges from 14% to 45% in HIV-positive persons (reviewed in [1]). Several groups have reported beneficial effects of HPgV viremia on HIV disease progression, as indicated by higher CD4 cell counts, lower HIV viral loads, and longer AIDS-free survival times [2–6]. In contrast, loss of HPgV RNA is associated with accelerated HIV disease [3, 7, 8]. In vitro studies demonstrate that HIV replication is inhibited by HPgV co-infection in peripheral blood mononuclear cell cultures [2]. Nevertheless, this beneficial effect of HPgV has not been observed in all studies [7–11]. Other studies conducted during the highly active antiretroviral therapy (HAART) era observed that a complete virologic response was more frequent in patients co-infected with HPgV, independent of CD4 cell count and HIV RNA level, although this was not uniformly found [12–14].

Country-specific data on HPgV are available from 13 Sub-Saharan African countries; the majority reported prevalence data and/or analysis of HPgV diversity [15]. Data regarding the impact of HPgV on HIV disease in this region are limited. Among HIV-positive women in Gambia, HPgV had no significant impact on HIV load, CD4 cell count, or mortality [16]. However, in HIV-positive South Africans, HPgV co-infection was associated with higher CD4 cell counts and lower HIV viral loads before HAART initiation, as well as faster viral load declines during HAART [17]. In Uganda, HIV/HPgV co-infected participants experienced slower CD4 cell decline and increased survival compared with HIV-positive adults who were HPgV-negative [18]. These data suggest that the beneficial effect of HPgV is not limited to the HIV subtypes predominant in the United States and Western Europe. Therefore, in resource-limited settings in which many HIV-infected individuals may not have access to antiretroviral therapy, a better understanding of the anti-HIV effects of HPgV infection may ultimately result in novel therapeutic strategies. Botswana has one of the highest HIV prevalence rates in the world [19, 20]. Thus, we evaluated the impact of HPgV infection in a natural history cohort of HIV disease progression in Botswana.

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METHODS

Study Participants

In 2005, the Botsogo Study was established among HIV-infected antiretroviral therapy (ART)–naïve individuals in Gaborone, Botswana, to observe disease progression among individuals infected with HIV subtype C who did not qualify for ART according to the Botswana national guidelines ($CD4^+$ T cell count ≥200 per mm^3 and a World Health Organization clinical stage I or II) at the time of enrollment [21]. Exclusion criteria included any AIDS-defining illness requiring the initiation of HAART or previous ART use or exposure, except for use as part of the prevention of mother-to-child transmission program, the presence of an AIDS-related malignancy, patients requiring chronic corticosteroid use, less than 3 months postpartum, and/or participation in any study that provides immune-modulating agents. During follow-up, participants visited clinics quarterly, including 1 month after enrollment. The study was approved by the Human Research Development Committee at the Botswana Ministry of Health and Wellness (protocol number HRDC #00667) and the Harvard School of Public Health's Office of Human Research Administration (protocol number 10366-127).

Detection of HPgV and Classification of HPgV Genotype

A secondary analysis of HPgV was performed in a convenience sampling of individuals enrolled in the Botsogo Study. As reported previously [22, 23], viral RNA was extracted from serum with the QIAmp Ultrasens Virus Kit (QIAGEN, Valencia, CA). HPgV RNA was detected by amplification of the 5' untranslated region (UTR) with the antisense primer 5' – ATG CCA CCC GCC CTC ACC CGA A – 3' (nucleotides [nt] 494–473 according to GenBank accession number AY196904) and the sense primer 5' – AAA GGT GGT GGA TGG GTG ATG – 3' (nt 67–87) via OneStep RT-PCR (QIAGEN). Amplification conditions were 50°C for 59 minutes, 10 minutes at 94°C, then 35 cycles of 30 seconds at 94°C, 1 minute at 55°C, and 1 minute at 72°C, followed by 20 minutes at 72°C. First-round polymerase chain reaction (PCR) products were used in nested PCR with the antisense primer 5' – CCC CAC TGG TCY TTG YCA ACT C – 3' (nt 362–341) and sense primer 5' – AAT CCC GGT CAY AYT GGT AGC CAC T – 3' (nt 107–131). After 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C, PCR products were analyzed by agarose gel electrophoresis for the presence of a 256-nt band. Population-based sequencing of amplicons was conducted, and 5'UTR sequences were aligned with GenBank accession numbers U59540, U59543, U59549, and U59555 (genotype 1); HGU59518, D90600, HGU59534, and HGU59535 (genotype 2); U59538 and U59539 (genotype 3); AB018667 and AB021287 (genotype 4); AY949771, AF092894, LT009490, KC618398, KC618400, KC618401, AY032965, AF172508, and KP710606 (genotype 5); AB003292 and AF177619 (genotype 6); and HQ331234 and HQ331235 (genotype 7). Phylogenetic inference was performed using a Bayesian Markov chain Monte Carlo (MCMC) approach executed in the Bayesian Evolutionary Analysis by Sampling Trees v1.8.4 [24] with an uncorrelated log-normal relaxed molecular clock, generalized time reversible model, and nucleotide site heterogeneity estimated with a gamma distribution. MCMC analysis was run for a chain length of 1 000 000 000. All effective sample sizes were >200, indicating sufficient sampling. The maximum clade credibility tree was selected from the posterior tree distribution after a 10% burn-in using TreeAnnotator v1.8.4. HPgV sequences were deposited in GenBank using accession numbers MF398545–MF398571.

Assessment of Liver Injury

The aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis 4 (FIB-4) score represent 2 noninvasive indices of liver damage (reviewed in [25]). APRI is equal to 100 * (AST/40) / platelet, while FIB-4 is calculated as age [years] × AST [IU/L] / √ (PLT [10^9/L] × (ALT [IU/L]). The APRI and FIB-4 indices were validated initially for hepatitis C virus and are now utilized during HIV mono-infection and chronic HBV as well [26–29].

Statistical Analysis

Sociodemographic and clinical data available at baseline were evaluated for the Botsogo Study. Fisher's exact test was used to evaluate the difference in proportions for dichotomous variables, and the Wilcoxon rank sum test was used to compare select categories. All statistical analyses were performed using STATA 14.1 (College Station, TX).

RESULTS

The Botsogo Study followed 436 participants for 5 years, of whom 356 (82%) were female [21]. The median age was 33 years (interquartile range [IQR], 27–39 years); 133 participants had baseline plasma available for the current analysis of HPgV. HPgV RNA was detectable in 41 (30.8%). HPgV-positive and HPgV-negative individuals did not differ with respect to age or gender (Table 1). ALT levels were lower for HPgV-positive compared with HPgV-negative individuals (15.4 vs 16.5 cells/uL; $P < .001$), although AST levels were not significantly different.

While HIV is known to impact liver disease progression in the presence and absence of viral hepatitis, the effect of HPgV infection on liver disease is unknown. Using 2 noninvasive indices of liver damage—APRI and FIB-4—there were no observed differences in liver disease between the 2 groups based on HPgV status. As shown in Figure 1A, the presence of HPgV RNA had no statistically significant impact on HIV viral loads. Baseline viral loads were 4.23 $\log_{10}$ copies/mL in HPgV-positive individuals and 4.15 $\log_{10}$ copies/mL in HPgV-negative individuals. However, the median CD4 cell count was higher for HPgV-positive compared with HPgV-negative individuals (589 cells/uL vs 501 cells/uL; $P = .0173$) (Figure 1B).

Table 1. Baseline Demographic and Clinical Data for HPgV-Positive and HPgV-Negative Individuals Enrolled in the Botsogo Study

	HPgV-Positive (n = 41)	HPgV-Negative (n = 92)	*P* Value
Age, median (Q1, Q3), y	34 (29, 41)	32 (28, 41)	.444
Male gender, n (%)	10/23 (43.5)	13/23 (56.5)	
Female gender, n (%)	31/110 (28.2)	79/110 (71.8)	.213*
Platelets, 10^9/L	267 (227, 310)	252 (224, 303)	.778
Hemoglobin, median (Q1, Q3), g/dL	12.8 (12.1, 13.7)	12.5 (11.3, 13.5)	.306
ALT , median (Q1, Q3), U/L	15.4 (12.3, 21.5)	16.5 (11.1, 24.6)	<.001
AST, median (Q1, Q3), U/L	22.0 (16.5, 27.3)	23.0 (18.3, 28.5)	.444
FIB-4 score, median (Q1, Q3)	0.71 (0.525, 0.931)	0.74 (0.58, 0.98)	.589
APRI score, median (Q1, Q3)	0.19 (0.15, 0.29)	0.22 (0.18, 0.31)	.342

The data represent medians (interquartile ranges in parentheses) except as noted. *Comparisons are made between the HPgV-positive and HPgV-negative groups using the Wilcoxon rank sum test, with the exception of male gender and HPgV status, for which the chi-square test was used.

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis 4.

HPgV genotypes were available for 27 individuals (65.9% of those with detectable HPgV RNA) and included 5 (18.5%) with genotype 1 and 22 (81.5%) with genotype 5 (Figure 2). Individuals with HPgV genotype 5 had significantly higher baseline CD4 cell counts than those with HPgV genotype 1 (617 cells/uL vs 428 cells/uL; $P = .0084$) (Figure 3).

DISCUSSION

These data represent the first study of HPgV prevalence conducted in Botswana. Data on HPgV are scarce in Sub-Saharan Africa and largely limited to small prevalence studies (reviewed in [15]). Cross-sectional studies conducted in South Africa demonstrate a prevalence range of 10.2% to 41.2% in blood donors, hemodialysis patients, transplant patients, hemophiliacs, or patients with chronic liver disease [30–34]. Thus, the current finding of an HPgV prevalence of 30.8% is in agreement with other studies conducted in southern Africa.

Multiple HPgV genotypes have been described at the population level [35, 36]. Genotypes 1 and 2 are common throughout the Americas and northern and central Africa. Genotypes 3 and 4 are present in Asia. Genotype 5 circulates within central and southern Africa. Genotype 6 has been identified in Southeast Asia, while a putative genotype 7 has only been reported in China [36]. This study is the first to evaluate HPgV genotypes in Botswana and suggests that genotype 5 is the predominant circulating genotype. HPgV genotype 5 has also been reported in Uganda, the Democratic Republic of the Congo, Tanzania, and Ethiopia [37–43]. In South Africa, HPgV genotype 5 is most common, although genotypes 1 and 2 have also been reported [34, 40–42, 44, 45]. However, several limitations require cautious interpretation of these findings, including a modest sample size and lack of genotype data for all HPgV-positive individuals. The lack of a statistically significant difference in HIV viral load by HPgV genotype could have been due to low sample size. As with other studies, there is no information about the mode of HPgV transmission or the timing of infection. While the correlation between HPgV RNA levels and CD4 cell count or HIV viral load was not evaluated in this analysis, previous studies have reported an inverse correlation between HPgV and HIV levels [4, 46].

The possible impact of distinct HPgV genotypes on HIV disease progression has been evaluated in other studies outside of Africa. For instance, Muerhoff et al. reported that CD4 cell

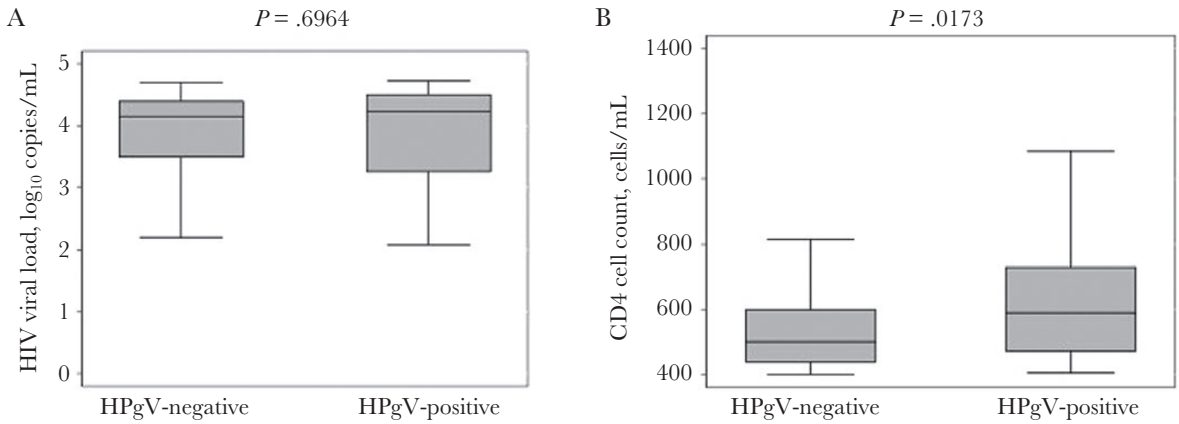

Figure 1. (A) Baseline HIV viral load (log_{10} copies/mL) and (B) CD4 cell count (cells/uL) were evaluated for human pegivirus (HPgV)–positive and HPgV-negative individuals. *P* values are shown for the Wilcoxon rank sum test.

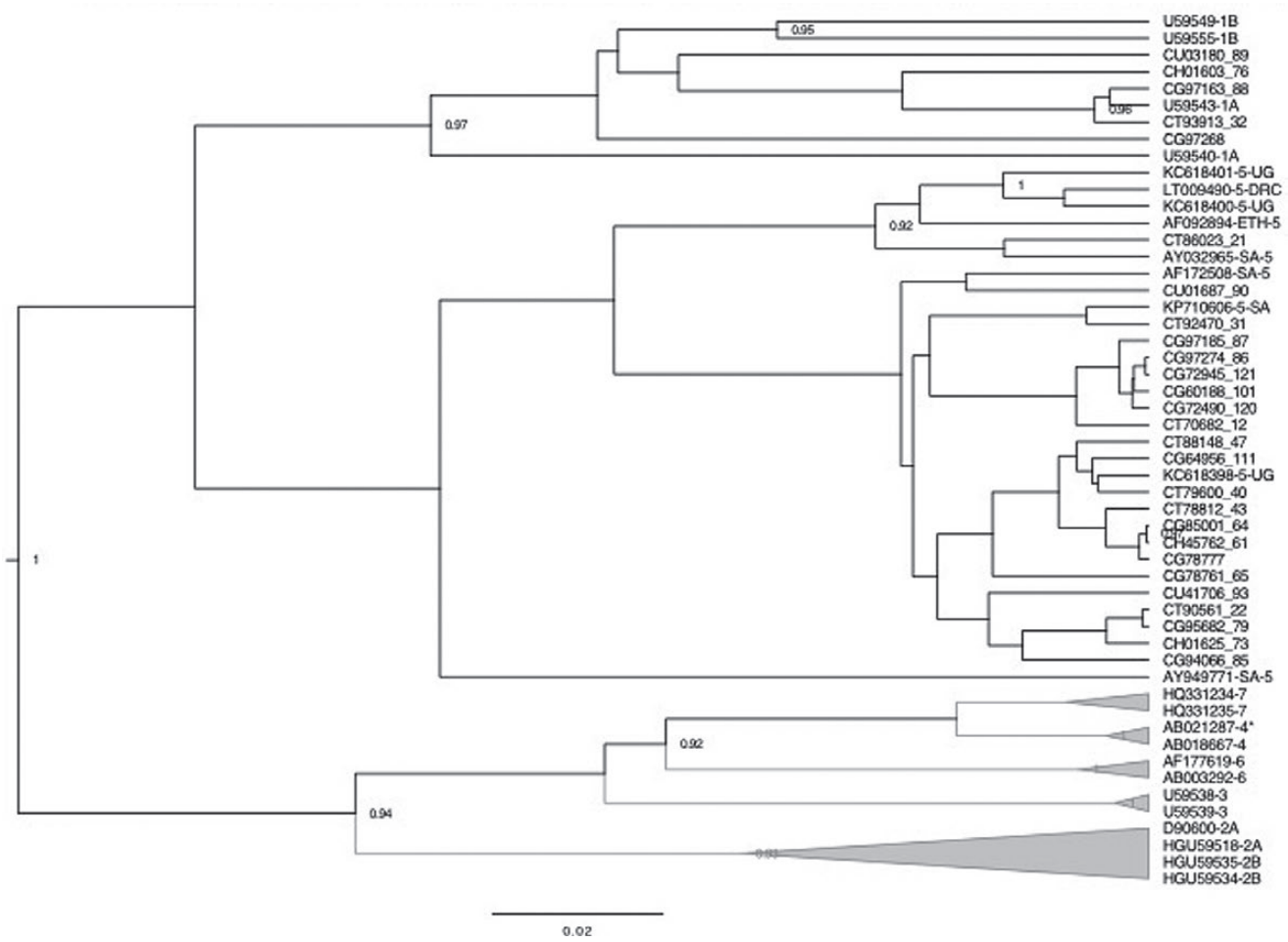

Figure 2. Bayesian phylogenetic analysis of the 27 HPgV 5′UTR sequences from this study (indicated by a 6-digit study ID + a 2- to 3-digit sequence number) compared with GenBank references (indicated by their accession numbers and genotype). Full-length HPgV sequences with evidence of recombination are denoted by an asterisk. Posterior probability values >0.90 are indicated at tree nodes.

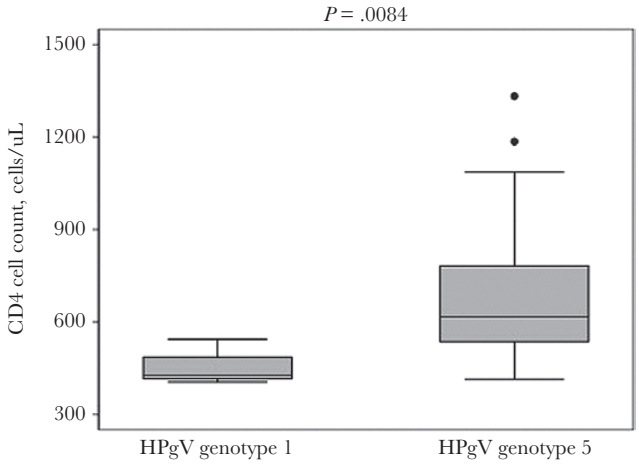

Figure 3. Baseline CD4 cell count (cells/uL) was evaluated for HPgV-positive individuals with genotypes 1 and 5. Closed circles represent outliers. The *P* value shown is for the Wilcoxon rank sum test.

counts tended to be lower in HIV-positive patients co-infected with HPgV genotype 2a compared with those with HPgV genotype 2b [47]. In US patients with HIV/HCV/HPgV triple infection, higher CD4 cell counts were associated with HPgV genotype 2 compared with genotype 1 [23]. Similar findings were observed in Brazil, although no difference in CD4 cell count based on HPgV genotype was reported in Australia [48, 49]. In Brazil, HPgV RNA levels also differed by genotype [50]. Unfortunately, studies designed to evaluate the potential influence of HPgV genotype on HIV disease progression have not been conducted in Africa to date. To date, only a single functional study has included HPgV genotype 5 isolates. Xiang et al. evaluated South African samples and found that genotype 1 and 5 isolates replicated in lymphocyte cultures, inhibited X4 and R5 HIV isolates, and induced the chemokines RANTES/CCL5 and stromal-derived factor–1 (SDF-1) in vitro [34]. However, too few HPgV isolates were included

to compare their ability to suppress HIV replication based on HPgV genotype.

The high prevalence of HPgV in Botswana, its beneficial impact on HIV disease progression, and the impact of HPgV genotype on CD4 cell count all suggest an immediate need to expand significantly the research on HPgV in resource-limited settings such as Sub-Saharan Africa.

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