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Citation
N’Guessan, K. F., M. Anderson, B. Phinius, S. Moyo, A. Malick, T. Mbangiwa, W. T. Choga, et al. 2017. “The Impact of Human Pegivirus on CD4 Cell Count in HIV-Positive Persons in Botswana.” Open Forum Infectious Diseases 4 (4): ofx222. doi:10.1093/ofid/ofx222. http://dx.doi.org/10.1093/ofid/ofx222.

Published Version
doi:10.1093/ofid/ofx222

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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.

Received 18 July 2017; editorial decision 4 October 2017; accepted 5 October 2017.

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DOI: 10.1093/ofid/ofx222
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 mm² 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 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 TTY 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-bp 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); ATY949771, AF092894, LT009490, KC618398, KC618400, KC618401, AYO32965, 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 [10^4/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 log10 copies/mL in HPgV-positive individuals and 4.15 log10 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).
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](image-url). (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.
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.

Acknowledgements

The authors would like to thank the study participants and staff of the Botso LGIC study, the Models of Care Project, sponsored by the Ministry of Health and Wellness and the African Comprehensive HIV/AIDS Partnerships (ACHAP), and the Pathobiology and Molecular Medicine PhD program at the University of Cincinnati College of Medicine for their generous support.

Financial support. The Botso LGIC study was funded by ACHAP, a country-led, public–private development partnership between the Government of Botswana, the Bill and Melinda Gates Foundation, and the MSD/Merck Company Foundation. The authors were supported by the Sub-Saharan Africa Network for TB/HIV Research Excellence (grant 107752/21/15/Z) from the Wellcome Trust, the Fogarty HIV Research Training Program for Low- and Middle-Income Country Institutions supported by the NIH Fogarty International Center (D43 TW009610), and the Oak Foundation (OUSA-12–025). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Potential conflicts of interest. All authors: no reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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