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High HIV-1 RNA Among Newly Diagnosed People in Botswana

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Abstract

HIV-1 RNA level is strongly associated with HIV transmission risk. We sought to determine whether HIV-1 RNA level was associated with prior knowledge of HIV status among treatment-naive HIV-infected individuals in Botswana, a country with high rates of antiretroviral treatment (ART) coverage. This information may be helpful in targeting HIV diagnosis and treatment efforts in similar high HIV prevalence settings in a population-based survey. HIV-infected individuals were identified during a household survey performed in 30 communities across Botswana. ART-naive persons with detectable HIV-1 RNA (>400 copies/mL) were divided into two groups, newly diagnosed and individuals tested in the past who knew about their HIV infection at the time of household visit, but had not taken ART. Levels of HIV-1 RNA were compared between groups, overall and by age and gender. Among 815 HIV-infected ART-naive persons with detectable virus, newly diagnosed individuals had higher levels of HIV-1 RNA ($n = 490$, median HIV-1 RNA 4.35, interquartile range (IQR) 3.79–4.91 $\log_{10}$ copies/mL) than those who knew about their HIV-positive status ($n = 325$, median HIV-1 RNA 4.10, IQR 3.55–4.68 $\log_{10}$ copies/mL; p values <.001, but p value = .011 after adjusting for age and gender). A nonsignificant trend for higher HIV-1 RNA was found among newly diagnosed men 30 years of age or older (median HIV-1 RNA 4.58, IQR 4.07–5.02 $\log_{10}$ copies/mL vs. 4.17, 3.61–4.71 $\log_{10}$ copies/mL). Newly diagnosed individuals have elevated levels of HIV-1 RNA. This study highlights the need for early diagnosis and treatment of HIV infection for purposes of HIV epidemic control, even in a setting with high ART coverage.

Keywords: HIV-1 RNA, HIV transmission, new HIV diagnosis, newly diagnosed males, Botswana

Introduction

HIV-1 RNA LEVEL is strongly associated with HIV transmission risk.[1–7] During the early stage of HIV infection in particular, high HIV-1 RNA load increases the risk of virus transmission.[8–12] Some individuals maintain high levels of HIV-1 RNA for an extended period of time[13,14] and could contribute disproportionately to new HIV transmissions. In contrast, low levels of HIV-1 RNA are associated with lower virus transmission, which provides a strong rationale for early antiretroviral treatment (ART) as a highly efficacious intervention for preventing virus transmission in serodiscordant couples,[15,16] and in the general population.[17]

The benefits of knowing one's HIV status[18] are in line with the Joint United Nations Program on AIDS (UNAIDS) ''90-90-90'' targets.[19] Many individuals living with HIV remain unaware of their HIV infection, particularly during the early stage of disease. In the era of scale-up of universal ART

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programs in southern African countries, undiagnosed people living with HIV infection are likely to remain a major source of new HIV transmissions. To reach undiagnosed individuals, targeted interventions are needed.[20,21]

Levels of HIV-1 RNA among undiagnosed individuals are unknown. Community viral load (VL) over time has been suggested as a population-level biomarker for monitoring of public health interventions.[22–24] However, community VL is calculated from surveillance data of reported cases,[25,26] and is associated with substantial limitations, as undiagnosed individuals living with HIV are not captured by this approach.

Botswana suffers from a severe, generalized HIV epidemic with estimated adult prevalence of 22%[27] and HIV incidence above 1%.[28,29] However, Botswana has achieved very high coverage of the UNAIDS ''90-90-90'' targets (knowledge of positive HIV status, sustained receipt of ART, and virological suppression after ART initiation).[30]

To test whether levels of HIV-1 RNA among HIV-infected ART-naive individuals differ between newly diagnosed and people tested in the past (who already knew about their HIV infection) or by age or gender, we utilized extensive data collected during a population-based household baseline survey in the Botswana Combination Prevention Project (BCPP), a community-randomized clinical trial in 30 communities across Botswana.[28,30–32] We focused our analysis on HIV-infected ART-naive individuals with HIV-1 RNA >400 copies/ml, given the strong scientific evidence that higher levels of HIV-1 RNA are associated with higher rates of virus transmission,[1–7] and the public health relevance of identifying subpopulations with higher levels of HIV-1 RNA, with regard to considering interventions most likely to lead to HIV epidemic control.

Materials and Methods

Study subjects

Study population and sampling procedures are presented elsewhere.[31] Briefly, the BCPP (the *Ya Tsie* study) is a pair-matched, cluster-randomized clinical trial in 30 communities across Botswana. The study assesses reduction in the cumulative HIV incidence as a result of combination prevention interventions and measures population-level uptake of HIV testing and ART.

A random sample of ~20% of residents (12,610 individuals) was enrolled during the baseline cross-sectional household survey from November 2013 to 2015. The HIV-positive status of participants 16–64 years of age was based on either written documentation provided, such as HIV test result, or ART prescription, or rapid HIV testing performed in the household according to the Botswana national guidelines. The majority of HIV-positive study participants, 83%, knew their HIV status.[31] Among those who knew their HIV status, 87% were receiving ART.[31] Based on the history of previous HIV testing (if any), HIV-positive ART-naive people were divided into two groups: newly diagnosed individuals, and individuals tested in the past who knew about their HIV infection at the time of the household visit.

HIV testing

Parallel HIV rapid testing was performed in households according to the Botswana government algorithm for HIV testing and included KHB (KHB, Shanghai Kehua Bio-Engineering Co. Ltd, Shanghai, China) and Uni-Gold (Trinity Biotech Plc, Bray, Ireland) assays.

HIV-1 RNA testing

HIV-1 RNA load in plasma was quantified in all enrolled individuals living with HIV infection regardless of their ART status. Venous blood was collected by phlebotomy in households and was processed in the mobile clinic within 4 h from sampling. The HIV-1 RNA load in plasma was quantified by Abbott m2000sp/Abbott m2000rt (Wiesbaden, Germany) at the Botswana–Harvard HIV Reference Laboratory, which is ISO 17025 accredited.

Cross-sectional estimation of HIV incidence

To identify individuals with recent HIV infection from cross-sectional sampling, HIV incidence was estimated as described elsewhere.[28,29] The algorithm for estimation of HIV recency-combined Limiting Antigen Avidity Assay (LAg-Avidity) data, ART status, and HIV-1 RNA load, as described in.[33] The LAg-Avidity threshold of normalized optical density was 1.5. Documented ART status was used as an exclusion criterion for HIV recency. The HIV-1 RNA threshold was 400 copies/mL. The Mean Duration of Recent Infection was 130 days[34] and the False Recent Rate was zero.[33] A total of 34 individuals were identified with recent HIV infection.

Statistical analyses

For heterosexual HIV transmission, there appears to be a viral RNA load threshold (i.e., 50–1,000 copies/ml), below which transmission is very unlikely to occur. Above such a threshold, transmission risk is clearly associated with increase in HIV-1 RNA load.[1,2] In this study, we focused on subjects whose HIV-1 RNA was above 400 copies/ml, although transmission below other thresholds (e.g., 1,000 copies/mL) may also be rare.

We began with an analysis of the extent to which effects of knowledge of positive HIV status on VL are mediated through ART use based on all HIV-infected patients, regardless of their VL or ART use. We then conducted analyses on all ART-naive subjects and finally focused on a subgroup of those with VL >400 copies/ml.

All confidence intervals (CIs) of estimated proportions are asymptotic 95% binomial CIs (95% CI). Comparisons of HIV-1 RNA levels between groups were performed using Wilcoxon rank sum test without and with adjusting for clustered communities (by using R package clusrank[35,36]). Mixed effects model with a random intercept to model between communities' variability were used with and without adjustment (gender and age groups 16–29 years of age, 30–44 years of age, and 45–64 years of age, and their interactions) to quantify the effects on HIV-1 RNA levels of being newly diagnosed and already knowing one's HIV status. When interactions were not significant they were removed from the model.

p Values were considered statistically significant if lower than .05 once using a Bonferroni procedure for multiple testing, where the p value is divided by the number of tests performed.[37] All 95% CI and statistical testing accounted for clustering by bootstrapping community data using random

Table 1. HIV-1 RNA in Individuals not Currently on Antiretroviral Treatment and with Detectable HIV-1 RNA (>400 Copies/mL), Median (Interquartile Range) $\log_{10}$ Copies/mL

	Previously diagnosed		*Newly diagnosed*		*Wilcoxon rank sum test, p value (adjusted p value)*[a]	*Non-adjusted analysis, mixed effect models*		*Adjusted analysis, mixed effect models*	
Group	*n*	*HIV-1 RNA*	*n*	*HIV-1 RNA*		*Difference*[b] *(SD)*	p	*Difference*[b] *(SD)*	p
ART Naive	379	3.897 (3.24–4.59)	574	4.158 (3.31–4.82)	.007 (.183)	0.14 (0.07)	.030	0.05[c] (0.07)	.257
ART Naive >400 copies/mL	325	4.10 (3.55–4.68)	490	4.35 (3.79–4.91)	.0003 (.002)	0.18 (0.06)	.001	0.13[d] (0.06)	.011

[a]Adjusted for community clustering.
[b]HIV-1 RNA point estimate difference between previously diagnosed and newly diagnosed individuals.
[c]Adjusted by sex, age group (16–29 years of age, 30–44 years of age, and 45–64 years of age), and sex–age group interaction.
[d]Adjusted by sex and age group (16–29 years of age, 30–44 years of age, and 45–64 years of age).
ART, antiretroviral treatment; SD, standard deviation.

Table 2. HIV-1 RNA in Males and Females not Currently on Antiretroviral Treatment and with Detectable HIV-1 RNA (>400 Copies/mL), Stratified by Age Group, Median (Interquartile Range) $\log_{10}$ Copies/mL

	Previously diagnosed		*Newly diagnosed*		*Wilcoxon rank sum test, p value (adjusted p values)*	*Non-adjusted analysis, mixed effect models*		*Adjusted analysis, mixed effect models*	
Age group	N	*HIV-1 RNA*	N	*HIV-1 RNA*		*Difference*[a] *(SD)*	p	*Difference*[a] *(SD)*	p
All males	64	4.271 (3.70–4.75)	190	4.568 (4.05–5.03)	.017 (.096)[b] (.034)[c]	0.24 (0.11)	.013 (.026)[d]	0.24[c] (0.11)	.016 (.032)[d]
Males 16–29 years of age	11	4.65 (4.25–4.97)	34	4.43 (3.94–5.08)	.58 (.897)[b] (1.00)[e]	–0.19 (0.29)	.250 (1.00)[e]	—	—
Males 30–44 years of age	36	4.20 (3.60–4.72)	122	4.61 (4.08–5.01)	.028 (.082)[b] (.168)[e]	0.285 (0.14)	.023 (.138)[e]	—	—
Males 45–64 years of age	17	4.12 (3.75–4.67)	34	4.49 (4.07–5.05)	.062 (.138)[b] (.372)[e]	0.44 (0.21)	.019 (.114)[e]	—	—
All females	261	4.089 (3.51–4.64)	300	4.200 (3.61–4.76)	.106 (.154)[b] (.212)[e]	0.10 (0.07)	.068 (.136)[d]	0.09[c] (0.07)	.081 (.162)[d]
Females 16–29 years of age	66	4.09 (3.62–4.65)	111	4.01 (3.62–4.52)	.95 (.983)[b] (1.00)[e]	0.03 (0.11)	.413 (1.00)[e]	—	—
Females 30–44 years of age	134	4.02 (3.50–4.57)	107	4.23 (3.54–4.77)	.26 (.148)[b] (1.00)[e]	0.10 (0.10)	.158 (.948)[e]	—	—
Females 45–64 years of age	61	4.19 (3.64–4.77)	82	4.31 (3.77–4.93)	.29 (.633)[b] (1.00)[e]	0.16 (0.13)	.120 (.720)[e]	—	—

[a]HIV-1 RNA point estimate difference between previously diagnosed and newly diagnosed individuals.
[b]Adjusted for community clustering.
[c]Adjusted by age group (16–29 years of age, 30–44 years of age, and 45–64 years of age) and sex–age group interaction.
[d]Bonferroni adjusted *p* value for four tests due to stratification by gender.
[e]Bonferroni adjusted *p* value for 12 tests due to stratification by gender and age.

sampling with replacement. Statistical analyses were performed in R version 3.3.1.[38]

Results

We performed an analysis of the extent to which ART treatment mediates the impact of knowledge of positive HIV status on VL[39] based on the entire population of HIV-positive individuals ($n = 3,596$). This analysis suggests that, as expected, ART has a large effect on VL; no unmediated effect of knowledge of HIV status on VL could be detected (results not shown). Restricting analysis to ART-naive individuals ($n = 953$) demonstrated that newly diagnosed individuals ($n = 574$) had higher levels of HIV-1 RNA than people who already knew about their HIV-positive status ($n = 379$; by 0.14 $\log_{10}$ copies/ml, standard deviation (SD) = 0.07; $p = .030$; Table 1), although the difference was nonsignificant after adjusting for age and sex (by 0.05 $\log_{10}$ copies/mL SD = 0.07; $p = .257$).

To focus on the subgroup believed to be capable of transmitting virus, the subsequent analysis was restricted to ART-naive individuals with HIV-1 RNA >400 copies/ml ($n = 815$). Such analyses do not have any causal interpretation, but are relevant only for describing the HIV-infected ART-naive population. Newly diagnosed individuals ($n = 490$) had higher levels of HIV-1 RNA than people who already knew about their HIV-positive status ($n = 325$) at the time of the household survey (Table 1; p value .0003–.011). Adjusted analysis showed that HIV-1 RNA may be increased by 0.13 $\log_{10}$ copies/ml (SD = 0.06; $p = .011$) in newly diagnosed individuals. The interaction tests, between age and knowledge of HIV status, and between sex and knowledge of HIV status, were not significant.

To describe subpopulations with elevated levels of HIV-1 RNA, we stratified data by gender and three age groups, 16–29 years of age, 30–44 years of age, and 45–64 years of age. We found a nonsignificant trend for higher levels of HIV-1 RNA in newly diagnosed males 30 years of age or older (Table 2; Wilcoxon rank sum test $p = .168$ for age 30–44 years of age and $p = .372$ for age 45+ years of age; the trend was confirmed by mixed effects model).

Early HIV infection is known to be associated with high HIV-1 RNA. However, among 490 newly diagnosed individuals, only 5% (95% CI 3%–7%) had recent HIV infection in a cross-sectional testing.

Discussion

This study suggests that there is a difference in HIV-1 RNA levels between newly diagnosed individuals and those already aware of their HIV status. There is a modest, nonsignificant trend for males 30 years of age or older to have higher levels of HIV-1 RNA. In this study, newly diagnosed individuals with high HIV-1 RNA load were identified during a population-based household survey in a large random sample of residents of rural communities across Botswana. Generalizability of the study findings and utilization of new HIV diagnosis as an indicator of higher HIV-1 RNA load (particularly among middle-aged and older males) require confirmation in other settings.

The study highlights the importance of the first ''90'' in the global UNAIDS targets.[19] Because high levels of HIV-1 RNA are associated with increased risk of HIV transmission,[2,40] targeted interventions that are able to reach and bring to care subpopulations who are unaware of their HIV infection (men in particular) are urgently needed in the region.

Men have less opportunity for HIV testing. As compared with women, men visit clinics less frequently and their chances to be approached for opt-out HIV testing are relatively low. A large proportion of men could not be reached for testing in households compared with women during our recent study in Mochudi, Botswana.[41] Corroborating our results, a recent study in KwaZulu-Natal, South Africa, reported that males were disproportionately untested, unaware of their HIV-positive status, untreated, and virologically unsuppressed.[42] A lack of awareness of HIV status was also associated with higher odds of detectable HIV-1 RNA in Kenya.[43]

The phenomenon of elevated HIV-1 RNA in newly diagnosed individuals could have several explanations. We found that only 5% of newly diagnosed individuals were in the early stage of HIV infection that is associated with higher HIV-1 RNA levels.[8–12] This suggests that undiagnosed individuals who maintain high levels of HIV-1 RNA after the acute HIV infection[13,14,44] could at least partially explain the observed phenomenon. Among individuals who were previously diagnosed with HIV, it is likely that those with higher HIV-1 RNA load experienced more rapid CD4 decline, started ART, and were therefore removed from the population of previously diagnosed individuals (which could be enriched over time by ''elite'' and viremic controllers[45–49]).

Therefore, the observed phenomenon could be explained, at least in part, by fewer disease progressors and accumulation of nonprogressors in this population of individuals diagnosed in the past. It is known that levels of HIV-1 RNA can vary by gender: in children, HIV-1 RNA is higher in boys than in girls,[50] and adult males have higher levels of HIV-1 RNA than women do in the early stages of HIV infection[51–57] (although the gender difference disappears in advance-stage disease[58–61]). The findings in this study could also reflect an earlier stage of HIV infection in newly diagnosed individuals versus those with known HIV status.

Adjusting for age and gender resulted in reduced significance for comparison of HIV-1 RNA between groups of newly and previously diagnosed individuals. Multiple factors could contribute to the observed reduction. By fitting models with more covariates, we add uncertainty. Age and gender may explain some of the effects of interest because they may confound the relationship of interest, that is, gender might be associated both with awareness of HIV status and levels of HIV-1 RNA. It could also indicate the uncertainty of the HIV infection stage within the groups of newly and previously diagnosed participants, which could lead to a limited prognostic value of HIV-1 RNA (time of infection was not available in this study).

Novel strategies able to reach undiagnosed individuals are urgently needed. When new HIV infections are identified, particular effort should be made to bring these individuals to care and initiate ART promptly. If the results of this study are confirmed by others, a particular focus in the region should be placed on subpopulations of newly diagnosed men 30 years of age or older, as their contribution to HIV transmission could be high due to elevated levels of HIV-1 RNA.

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Authors' Contributions

V.N.: Conceived the project, analyzed data, organized, and wrote the article; M.P.: Performed biostatistical support of the project and data analysis; S.M.: Responsible for all laboratory data, including testing for HIV-1 RNA load; T.G.: Led the field team and data collection across 30 communities in Botswana; M.M.: Secured community support and set up field teams across 30 communities in Botswana; E.K.Y.: Coordinated field team efforts and data collection, performed data cleaning; U.C.: Responsible for specimens and data collection, supervised field teams; R.L.: Coordinated project with Botswana Ministry of Health; N.K.: Cleaned project data, maintained the project database, and provided critical support during data analysis; K.M.P.: Conceived key parts of the project, wrote, and edited the article; E.V.W.: Responsible for all data collection, storage, transfer, and maintenance of the project database; S.G.: Led and guided all laboratory testing in the project, and analyzed data; S.D.-P.: Provided critical review of project, performed data analysis, wrote and edited the article; M.P.H.: Administered the project, supervised obtaining of necessary approvals, coordinated interactions between Botswana and Harvard teams; V.G.: Led all statistical analysis; P.B.: Coordinated project with CDC, edited the article; J.M.: Guided coordination among clinical teams, laboratory, and data center; S.L.: Conceived key parts of the project, provided critical edits to the article; M.E.: Conceived the project, guided the analysis, provided critical edits to the article.

Disclosure Statement

No competing financial interests exist.

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