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Prevalence and risk of residual viremia after ART in low- and middle-income countries

A cross-sectional study

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Abstract

In order to design effective strategies to eradicate the HIV, an understanding of persistent viral reservoirs is needed. Many studies have demonstrated HIV residual viremia prevalence in high income countries, data from low- and middle-income countries (LMIC) are limited. We assessed the prevalence, and factors associated with residual viremia in people with HIV (PWH), who were virally-suppressed on antiretroviral therapy (ART) in LMIC. We also compared residual viremia prevalence between the LMIC and US.

This is a cross-sectional, retrospective study that utilized stored specimen samples from the AIDS clinical trials group (ACTG) studies A5175 and A5208. The last available sample among participants with plasma HIV RNA < 400 copies/mL for ≥3 years were tested by the HIV molecular and monitoring core gag (HMMCgag) single copy assay (SCA). Residual viremia was defined as detectable if ≥1 copy/mL. Spearman's correlation and multivariable stepwise logistic regression were used to assess associations of various factors with SCA.

A total of 320 participants, 246 (77%) from LMIC and 74 (23%) from US, were analyzed. Median (IQR) age was 33 (2840) years; baseline CD4 166 (88,230) cells/mm^3; HIV RNA 5.0 (4.5, 5.3) log10 copies/mL; duration of viral suppression 3.4 (3.1, 4.0) years and 48% were male. In 85 participants with information available, 53% were subtype C, 42% subtype B and 5% other subtypes. Overall prevalence of residual viremia was 57% [95% CI, 52–63] with 51% [40–63] in US and 59% [53–65] in LMIC. Among participants with detectable SCA, the median (IQR) HIV RNA was 3.8 (2.2, 8.1) copies/mL. The multivariable model conducted in LMIC participants showed that higher baseline HIV RNA was associated with detectable residual RNA (OR 2.9, 95% CI 1.8, 4.6 for every log10 increase, $P < .001$). After including both US and LMIC in the final model, baseline HIV RNA remained significant. No difference in SCA detestability was found between US and LMIC sites (OR 1.1 [0.6, 2.0], $P = .72$) after adjusting for baseline RNA and parent study.

The prevalence of residual viremia between both groups were not different and more than half of the participants had detectable viremia. Higher baseline HIV RNA was independently associated with residual viremia.

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Abbreviations: ACTG = AIDS clinical trials group, ART = antiretroviral therapy, HMMCgag = HIV molecular and monitoring core gag, INSTI = integrase strand transfer inhibitor, LMIC = low- and middle-income countries, NNRTI = non-nucleoside reverse transcriptase, PI = protease inhibitor, PWH = people with HIV, SCA = single copy assays.

Keywords: HIV-1, HIV molecular and monitoring core gag single copy assay, lowand middle-income countries, prevalence, residual viremia, risk factors

1. Introduction

For the past decade, antiretroviral therapy (ART) has successfully reduced the replication of HIV and significantly improved the survival of people with HIV (PWH). However, life-long suppressive therapy is still required. In order to design effective strategies to eradicate the HIV, an understanding of persistent viral reservoirs is needed. A recent study demonstrated that HIV reservoirs persisted in multiple body compartments such as gut and lymphoid tissues; blood in particular was the main source of dispersal.[1] HIV can persist latently but is inducible in resting memory CD4 T cells.[2,3] Multiple studies investigating the HIV reservoir have reported that residual viremia was detectable at a high prevalence (62%–77%) despite many years of viral suppression.[4–6] Most studies focusing on HIV reservoir prevalence were done in high income countries, despite that the majority of HIV infected individuals live in low- and middle-income countries (LMIC). Therefore, data regarding HIV reservoirs from LMIC are limited, and may indeed differ from high income countries. LMIC frequently have large populations living in overcrowded conditions and have low socioeconomic status (SES) included education and income. Thus, people living in LMIC may have high risk for others communicable diseases such as tuberculosis, viral hepatitis B, viral hepatitis C, sexually transmitted diseases and tropical disease infections that may negatively impact immunological and virological responses.[7–9] Furthermore, differences between HIV subtypes may affect the immunological progression of HIV through different interactions with the host cells.[2,10,11] Additionally, in vitro evidence suggests that latency mechanisms may depend on HIV subtypes.[12]

One of the originally described "single copy assays" (SCA) was designed to detect as little as one copy of HIV RNA/mL of plasma by qRT-PCR, providing significant insights into HIV persistence despite potent ART. In addition, it allows for a more thorough exploration of the impact of different therapeutic strategies on residual viremia. The originally described assays focused on analysis of clade B viruses.[13,14] A subsequently developed HIV molecular and monitoring core gag (HMMCgag) assay[15] is a multiplexed assay designed to detect nearly all non-B and B-clade pre-therapy patient samples, including many specimens that were previously missed by gSCA.[14]

We evaluated the prevalence of residual viremia, defined as detectable (≥1 copy/mL) HIV RNA by the HMMCgag assay, among HIV-infected participants on ART with virological suppression living in LMIC and compared with those from US. We used the stored samples from AIDS clinical trials group (ACTG) A5175 and A5208 studies. We also assessed factors associated with residual viremia.

2. Methods

2.1. Study population

This is a cross-sectional, retrospective study that utilized stored specimen samples from A5175 and A5208. The study population, trial design and study findings of ACTG studies A5175 and A5208, registered at www.ClinicalTrials.gov as NCT00084136 and NCT00089505, have previously been described.[16,17] In brief, the A5175 was a randomized controlled trial (Prospective Evaluation of Anti-retroviral Combinations for Treatment Naive, HIV Infected Persons in Resource-limited Settings: PEARLS) that enrolled 1571 participants from 8 low-middle-income countries (Thailand, Haiti, India, Malawi, Peru, Zimbabwe, Brazil and South Africa) and the United States, and prospectively evaluated the efficacy of protease inhibitor- (PI) and non-nucleoside reverse transcriptase (NNRTI)-based regimens as initial treatment for PWH.[16] Specimens for this study were collected between 2005 and 2007. The A5208 (Antiretroviral Therapies in Women after Single-Dose Nevirapine Exposure: OCTANE) compared efficacy of nevirapine versus lopinavir/ritonavir in women previously exposed to single-dose nevirapine in in 7 African countries (South Africa, Kenya, Zimbabwe, Botswana, Zambia, Malawi, and Uganda).[17] Specimens for this study were collected between 2005 and 2008. LMIC was defined by the world bank' classification, based on gross national income.

Participants were selected for this study if they had stored pre-ART samples, and a second sample after having been on ART with plasma RNA < 400 copies/mL for more than 3 years (approximately 144 weeks). Plasma HIV-1 RNA for the studies was measured in real time by the Roche Amplicor Monitor assay (v1.5) at laboratories participating in the US NIAID Division of AIDS Virology Quality Assurance Programs. Single blips followed by another value below 400 copies/mL were allowed. Written informed consent was obtained from all participants when the parent study was conducted. This study was approved by relevant IRBs for each site.

2.2. Single-Copy HIV RNA assay for residual viremia

All eligible samples were tested by the HMMCgag assay. The assay measures a region in HIV gag and has been described in a previous study.[15] Plasma was stored at −80°C prior to batch testing. The detection limit with HMMCgag assay is dependent on the volume of plasma analyzed, which is based on the minimum detectable 1.3 copies per sample divided by the plasma volume. The plasma volume range was 1.0 to 2.6 mL. The detection limit ranges from 0.5 to 1.3 copies/mL. We used the cut-off of 1 copy/mL to define the residual viremia. If HIV RNA in the last sample after viral suppression was below the limit of detection for the HMMCgag assay, the pre-ART sample was assessed to ensure that the assay detected the participant's virus, to reduce the risk of bias of false negative results due to sequence mismatches.

2.3. Statistical analysis

The prevalence of HIV RNA by HMMCgag assay ≥1 copy/mL was estimated as a binomial proportion along with a two-sided 95% confidence interval. Comparison of prevalence between

LMIC and US was done by Fisher exact test; comparison of prevalence within LMIC was undertaken by Chi-Square test. In analyzes to assess associations between HMMCgag positivity and various baseline and on study factors, HMMCgag results were evaluated as both continuous and dichotomized outcomes (HMMCgag <1 vs ≥1 copy/mL). Rank-based Spearman correlations were used to assess associations between 2 continuous variables and Wilcoxon rank-sum test and Kruskal–Wallis test were used between a continuous variable and a categorical variable. Adjustment for additional factors were done using stratified Wilcoxon rank-sum test and partial Spearman correlations. When HMMCgag results were considered as a continuous outcome, results below the lower limit of detection were imputed with the value of half of the detection limit.

Multivariable logistic regression was used to assess the association of various factors with residual viremia, defined as HMMCgag ≥1 copy/mL. Significant factors with a p-value <0.2 in univariable logistic regression were entered into an initial multivariate logistic model. Stepwise selection was then used to derive the final multivariable logistic model. All statistical tests were two-sided, at a nominal 5% significance level, and no adjustment was made for multiple comparisons. The participants with missing data were not included in the analysis. The sensitivity analysis, which was restricted to A5175 participants from both LMIC and US, were performed in the final model.

Sample size calculation is based on a cross-sectional study design. For the primary objective, assuming the prevalence HIV reservoirs detectable by SCA is 66%.[14,15] and approximately 25% of the samples will be excluded from the analysis due to primer mismatch and other assay/shipping issues, the width of a two-sided 95% confidence interval equals to 0.094.

3. Results

3.1. Participant characteristics

A total of 320 participants were included in the analysis. Participant characteristics overall and by LMIC and US sites are shown in Table 1. Two hundred forty six (77%) participants were from LMIC sites and 74 (23%) participants were from US

Table 1

Participant baseline characteristics, by site location.

	Site location			
	Total (N = 320)	**US (N = 74)**	**LMIC (N = 246)**	***P* Value**
Age (yr), Median (IQR)	33 (28, 40)	38.5 (30, 47)	32.5 (28, 38)	<.0001[a]
Female (n%)	168 (53%)	14 (19%)	154 (63%)	<.0001[b]
Race (n%)				<.0001[c]
• Black or African American	167 (52%)	29 (39%)	138 (56%)	
• White	64 (20%)	35 (47%)	29 (12%)	
• Other	89 (28%)	0 (0%)	87 (32%)	
Country (n%)				<.0001[c]
• Peru	81 (25%)	0 (0%)	81 (33%)	
• Malawi	55 (17%)	0 (0%)	55 (22%)	
• South Africa	42 (13%)	0 (0%)	42 (17%)	
• Brazil	42 (13%)	0 (0%)	42 (17%)	
• Botswana	25 (8%)	0 (0%)	25 (10%)	
• Kenya	1 (<1%)	0 (0%)	1 (<1%)	
• United States	74 (23%)	74 (100%)	0 (0%)	
Parent study (n%)				<.0001[b]
• A5175	252 (79%)	74 (100%)	178 (72%)	
• A5208	68 (21%)	0 (0%)	68 (28%)	
Baseline CD4 (cells/mm^3), Median (IQR)	166 (88, 230)	179 (71, 255)	162 (90, 224)	.43[a]
CD4 (cells/mm^3) at the time of SCA, Median (IQR)	472 (360, 614)	536 (389, 700)	457 (356, 588)	.01[a]
Baseline HIV-1 RNA (log_{10} copies/mL), Median (IQR)	5.0 (4.5, 5.3)	5.0 (4.6, 5.4)	4.9 (4.5, 5.3)	.58[a]
Category of baseline HIV-1 RNA (copies/mL), (n%)				.56[c]
• <100,000	172 (54%)	39 (53%)	133 (54%)	
• ≥100,000, <500,000	119 (37%)	26 (35%)	93 (38%)	
• ≥500,000	29 (9%)	9 (12%)	20 (8%)	
Baseline HIV Subtype (Total n = 85), (n%)				<.0001[c]
• AG	2 (2%)	2 (13%)	0 (0%)	
• B	36 (42%)	13 (87%)	23 (33%)	
• C	45 (53%)	0 (0%)	45 (64%)	
• Complex recombination	2 (2%)	0 (0%)	2 (3%)	
ART regimen at study entry				1.00[b]
• NNRTI	215 (67%)	50 (68%)	165 (67%)	
• PI	105 (33%)	24 (32%)	81 (33%)	
ART regimen at the time of SCA (n = 300)				.34[c]
• NNRTI	200 (63%)	47 (64%)	153 (62%)	
• PI	100 (31%)	25 (34%)	75 (30%)	
Co-infection* disease at the time of SCA (n%)	99 (31%)	21 (28%)	78 (32%)	.67[b]
Duration of viral suppression (yr), Median (IQR)	3.4 (3.1, 4.0)	4.4 (3.6,4.7)	3.3 (3.0,3.8)	<.0001[a]

[a] Wilcoxon Test.

[b] Fisher Exact Test.

[c] Chi-Squared Test.

* Co-infection disease includes hepatitis B, hepatitis C, tropical infection, TB and sexually transmitted diseases.

NNRTIs = non-nucleoside reverse transcriptase inhibitors, PI = protease inhibitors, SCA = single copy assay.

sites. Based on the parent study, 252 (79%) participants were from A5175 and 68 (21%) from A5208; all participants from US sites were from the A5175 study only. The median age for all study participants was 33 (interquartile range (IQR), 2840) years, and approximately half (53%) were female. Fifty two percent were black or African American, 20% were white, and 28% were other races. Among all participants, the median (IQR) CD4 cell count was 166 (88, 230) cells/mm^3 and 472 (360, 614) cells/mm^3 at study entry and at the time of HMMCgag assay testing, respectively. Participants from LMIC were younger and more likely to be female and black.

The median pre-ART HIV RNA was 5.0 (4.5, 5.3) $\log_{10}$ copies/mL. One hundred seventy two (54%) participants had a pre-ART HIV RNA <100,000 copies/mL, 119 (37%) were between 100,000 and <500,000 copies/mL, 29 (9%) were ≥500,000 copies/mL. The median pre-ART HIV RNA was similar between LMIC and the US groups (4.9 vs 5.0 $\log_{10}$ copies/mL; $P=.58$). The median duration of viral suppression was 3.4 (3.1, 4.0) years. Participants from LMIC had shorter duration of viral suppression compared to US participants (median 3.3 vs 4.4 years; $P<.0001$) and their CD4 cell count at the time of HMMCgag testing was lower (median 457 vs 536 cells/mm^3; $P=.01$).

Baseline ART regimens were NNRTI (67%) and PI (33%) -based regimens, and similar by site location groups. HIV genotyping was performed in 85 of 320 participants, 53% were subtype C, 42% subtype B, and 5% other subtypes.

3.2. Prevalence and associated factors of residual viremia

Overall prevalence of residual viremia was 57% [95% CI, 52–63] with 59% [53–65] in LMIC and 51% [40–63] in US ($P=.28$) (Fig. 1). Prevalence of residual viremia among participants in LMIC were Botswana (72%), Malawi (46%), Peru (54%), Brazil (52%), and South Africa (83%) ($P=.003$). Among participants with detectable SCA, the median (IQR) HIV RNA was 3.8 (2.2, 8.1) copies/mL. Levels of residual viremia among participants with detectable HMMCgag values by country are shown in Table 2.

Since the primary aim of this study was to assess factors associated with residual viremia in LMIC, we restricted this part of the analysis to LMIC participants only. Higher baseline RNA ($r=0.29$, $P<.001$) and shorter duration of viral suppression ($r=-0.19$, $P=.002$) were associated with higher HMMCgag HIV RNA, by Spearman correlation.

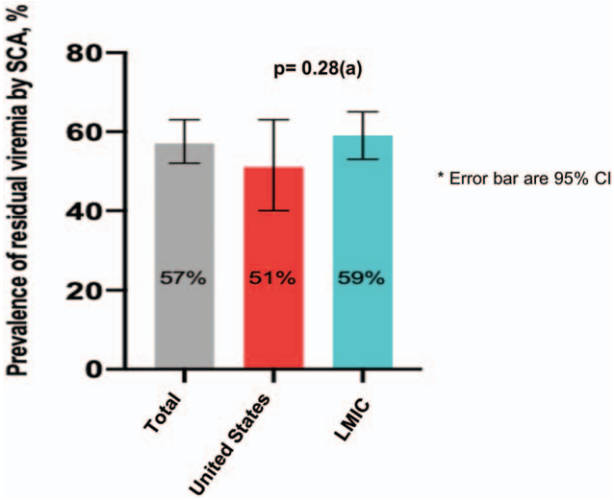

Figure 1. Prevalence of detectable residual viremia.

In univariable logistic regression analysis, baseline CD4 cell count, baseline HIV-RNA, duration of viral suppression, race, ethnicity, country and ART regimen at the time of HMMCgag testing had P values <.2, and entered into the multivariate model. In the final multivariable model, only higher baseline HIV RNA remained associated with detectable residual RNA by HMMCgag assay (aOR 2.9, 95% CI 1.8, 4.6 for every $\log_{10}$ increase, $P<.001$) after adjusting other covariates. Among participants from LMIC, residual viremia was no longer associated with duration of viral suppression. The forest plot of univariate and multivariate analysis for risk factors associated with residual viremia in LMIC are shown in Figure 2.

In sensitivity analysis, which was restricted to A5175 participants from both LMIC and US in the final model, baseline HIV RNA remained significantly associated with detectable residual RNA. There was no significant difference in HMMCgag assay RNA detectability between LMIC and US sites (aOR 1.1 [0.6, 2.0], $P=.72$) after adjusting for baseline RNA. In analyzes of the subset of participants with HIV subtype B or C from LMIC (N=68), we found no significant association between HIV subtype and detectable residual RNA by HMMCgag assay (OR 2.5 [0.3, 24.7], $P=.43$)

4. Discussion

Although previous studies have demonstrated clinically important associations with residual viremia in ART-treated individuals including chronic immune activation[18–20] and virological rebound risk after successful suppression,[21] data describing residual viremia in LMIC remains limited. Our study assessed the prevalence and risk factors associated with residual viremia in stored samples of 320 HIV infected participants from LMIC and US who participated in ACTG A5175 and A5208. The median duration of virological suppression in all participants was 3.4 years; 59% of participants from LMIC had detectable HIV RNA in plasma by HMMCgag assay. The prevalence of residual viremia did not differ significantly between LMIC and US participants (51%). This was confirmed by sensitivity analysis restricted to A5175 participants from both LMIC and US which showed no significant difference in HMMCgag assay RNA detectability. This prevalence was consistent with the previous reported residual viremia by the SCA of Palmer et al[14] from US (62%).[4] Despite the fact that LMIC participants have been on ART significantly shorter than US participants (3.3 vs 4.4 years, $P<.0001$), we were unable to detect a difference in the residual viremia between LMIC and the US. This can be explained by the slow rate of decay in residual viremia (6% per year) which was reported in a longitudinal residual viremia study.[22]

In multivariable analysis of risk factors associated with residual viremia, we found that higher baseline HIV RNA was associated with detectable residual RNA. Our findings are consistent with previous studies,[6,22–28] suggesting that the residual viremia is derived from cells which became latently infected before ART initiation. A recent study also confirmed that latent reservoirs were most genetically similar to viruses replicating just before ART initiation.[29] Furthermore, the lack of detectable HIV molecular evolution in the rebounding virus population after ART interruption[30] and the lack of impact of treatment intensification to suppressive regimens on residual

Table 2

Level of residual viremia and proportional of detectable residual viremia among participants -by country.

	Total (N = 183)	Country						*P* value
		Botswana (N = 18)	Brazil (N = 22)	Malawi (N = 26)	Peru (N = 44)	South Africa (N = 35)	US (N = 38)	
Level of residual viremia among participants with detectable by HMMCgag assay (copies/mL), Median (IQR)	3.8 (2.2, 8.1)	4.4 (2.9, 6.7)	3.2 (1.4, 6.9)	3.0 (1.5, 4.3)	5.4 (3.2, 9.3)	4.5 (1.7, 17.0)	3.9 (2.2, 7.9)	.11*
Proportional of detectable residual viremia by HMMCgag assay (≥1 copy/mL), % (95% CI)	57% (52–63)	72% (54–90)	52% (37–67)	46% (33–59)	54% (43–65)	83% (72–95)	51% (40–63)	.003**

* Kruskal–Wallis Test.
** Chi-Squared test.

viremia[6,31–40] suggest that the source of residual viremia is likely to be from latently infected cells. Given the similarity of our findings to multiple prior studies suggests that the source of residual viremia from PWH in LMICs also arises from the latent cell pool.[6,38–40]

We did not observe an impact of anchor agent (NNRTI or PI) on residual viremia consistent with previous studies.[22,28] On the other hand, many studies reported lower residual viremia on NNTRI-based as compared to PI based regimens.[41–45] Recently, integrase strand transfer inhibitor (INSTI) based regimens have been investigated in ART-switching clinical trials evaluating the effects of therapy on the HIV reservoir. However, the benefit of switching to INSTIs on residual viremia is still unclear. One study showed a significant decrease in HIV reservoir size, measured by total HIV DNA in peripheral blood after switching from NNRTI or PI to Raltegravir based regimens,[46] whereas many studies showed no impact of switching to INSTIs on residual viremia.[31,47–49]

We evaluated the association of sex with the residual viremia and did not see evidence of a difference in levels of low-level viremia between males and females. Sex was not significant in the univariate analysis, and was therefore not included in the final multivariable models, based on the pre-specified model selection process. In a multivariate model on sex adjusting for selected covariates such as baseline CD4, baseline HIV RNA and age (data not shown), no association between sex and residual viremia was detected. Previously, sex- based differences were detected[50] in a prospective study of low level viremia in 43 men and 41 women with subtype B HIV who were matched (1:1) for duration of suppression, CD4, and nadir CD4 cell counts. It is not clear why this association was not detected in the present analysis; our sample size of female participants who had

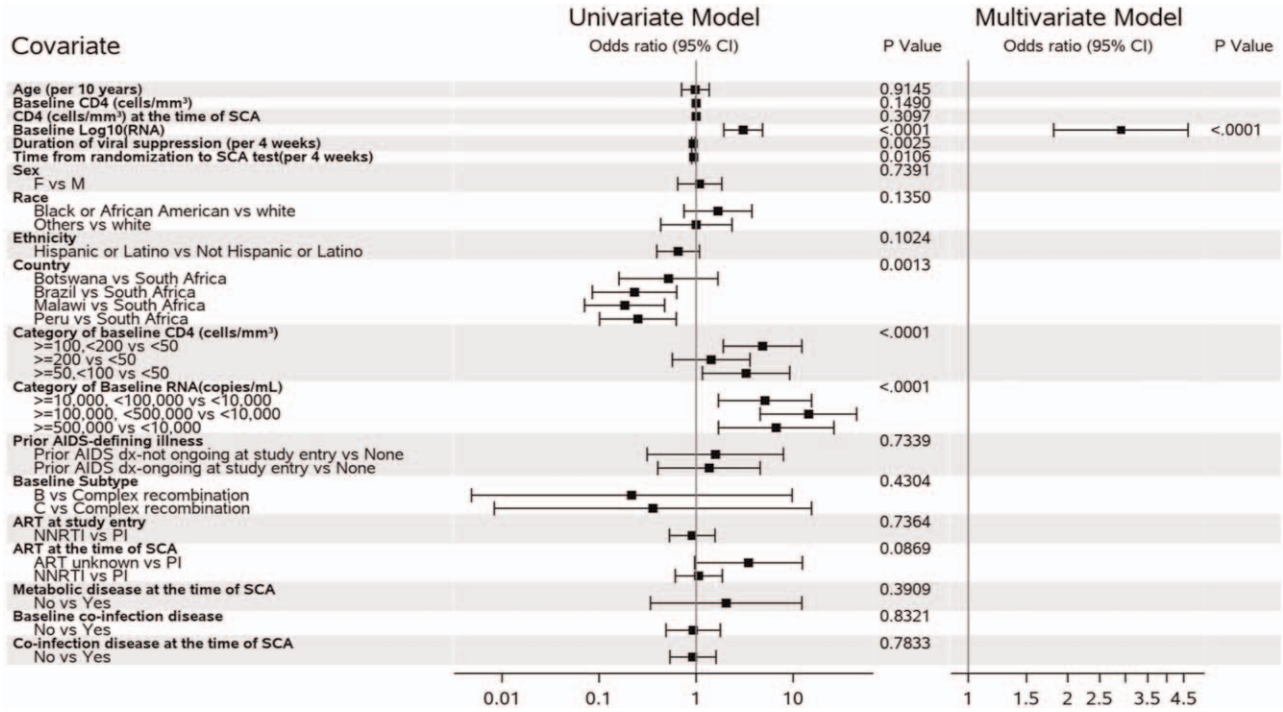

Figure 2. Forest plot of univariate and multivariate analysis for risk factors associated with residual viremia in LMIC.

detectable residual viremia (N = 92) is higher than previous studies. Additional studies will be essential to quantify sex-based differences in low level HIV viremia.

HIV subtypes are associated with different rates of AIDS progression[11] and CD4T cell decline.[51] In this study, the majority of US participants were infected with subtype B (87%) and LMIC participants were infected with subtypes C (64%) and B (33%). A previous study revealed that infection with subtype B was associated with fewer episodes of viremia detectable using a commercial assay under effective ART.[52] However, our study, using a single copy assay showed no association between HIV subtypes and residual viremia amongst those with subtype information available.

In our univariate analysis we found no association between residual viremia and age, prior AIDS defining illness, current underlying metabolic diseases or co-infection diseases (hepatitis B, hepatitis C, tropical infection, TB and/or sexually transmitted diseases). There are little previous data on the effects of co-infection on HIV viremia. A previous study of 14 individuals, who were studied early (6 months) after initiating ART with HIV RNA <1000 copies/mL, reported that HIV/TB co-infection was associated with increased markers of inflammation but not cell associated HIV RNA, when compared to HIV patients without TB infection.[53] In line with the current study that, the presence of active co-infection at the time of HMMCgag results were obtained was not associated with an increase in low level viremia. In addition, a previous study also demonstrated the lack of association between markers of inflammation and level of residual viremia in long-term ART participants.[28]

There are a number of limitations which limit the generalizability of our study findings. First, this is a cross-sectional study, which precludes analysis of longitudinal changes in each population. Secondly, all participants in this study were treated with NNRTI or PI based regimens, and samples were collected from 2005 to 2008. Many LMIC currently use INSTI as first line ART regimens. Third, although a sensitivity analysis restricted to A5175 participants from LMIC and the US found the prevalence of HMMCgag assay RNA detection in these settings was comparable, heterogeneity in assay detection across the LMIC was high. Fourth, previous studies[54,55] demonstrated a significant correlation between early ART initiation and a lower level of HIV reservoir among chronically HIV infected individuals. The majority of our participants initiated ART with advanced HIV (>50% of participants initiated ART with CD4 cell counts <200 cells/mm^3) whereas most HIV nowadays is diagnosed earlier, and ART is initiated soon after. Finally, HIV subtype was not available in every participant, which limits the power to demonstrate an impact of HIV subtype on HIV reservoir.

Despite these limitations, to our knowledge, this is the first study to compare residual viremia in long-term virally suppressed PWH in LMIC and US. The prevalence of residual viremia between both groups were not different and more than half of the participants had detectable viremia. Higher baseline HIV RNA was independently associated with residual viremia.

Further longitudinal research to investigate HIV reservoirs and new therapeutic procedures is warranted, in order to develop effective strategies to reduce or eliminate long-lived HIV reservoirs.

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