Demographic and clinical correlates of HIV-1 RNA levels in antiretroviral therapy-naive adults attending a tertiary hospital in Jos, Nigeria

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Abstract
Background: Plasma HIV-RNA viral load (VL) of HIV-infected persons is an important prognostic factor in HIV management. We determined the VL among antiretroviral therapy (ART)-naive patients to identify the association between patients’ demographic, clinical and laboratory characteristics with VL.

Method: A cross-sectional study of 224 ART-naive HIV-1-infected patients (≥15 years of age) accessing care at the Jos University Teaching Hospital AIDS Prevention Initiative in Nigeria ART treatment centre, from October 2010 to April 2011. A log-linear model was used to determine if VL was related to demographic and clinical variables.

Results: The patients had a median (interquartile range) age of 34 (28–41) years with females in the majority (59%). Females compared to males and pulmonary tuberculosis (PTB) co-infected compared to not co-infected patients had a significantly higher VL (14.9 log10, versus 11.5 log10, P=0.003 and 11.31 log10, versus 11.89 log10, P=0.047, respectively). VL tended to decrease with increasing CD4+ cell count levels in females, but remained relatively unchanged in males across all values of CD4+ cell counts. The difference (β) in the mean change in VL between males and females was loge 0.64 copies/mL, P=0.005.

Conclusion: In ART-naive HIV-1-infected patients in our setting, females had significantly higher VL and lower CD4+ cell count at the same VL threshold, compared to males, and hence were more likely to be at a higher risk of rapid progression to AIDS. Therefore, gender-based strategies for early identification and engaging females into care are required in this setting to mitigate against rapid progression to AIDS.

Keywords: HIV, viral load, CD4+ cell count, sex difference, ART naive

Introduction
Plasma HIV-1 RNA viral load (VL) levels of HIV-infected persons is a strong predictor of: sexual and perinatal transmission of HIV; rapidity of achieving viral suppression with antiretroviral therapy (ART) [1–3]; and the probability of progression to AIDS or death [4]. Pre-ART HIV-RNA VL levels can be exploited in the strategic timing of ART, particularly in the settings of limited resources [5]. An African study that modelled the relationship of VL and heterosexual transmission risk in discordant couples concluded that 90% of new HIV infections could be eliminated by treating only people with HIV VL >10,000 copies/mL [6].

Several factors are associated with VL in untreated patients: female sex [7,8], and a number of host factors such as HLA-B*5701, HLA-B*27, CCR5 delta-32 heterozygosity and allelic variation in HLA-C and KIR [9–11] have been shown to be associated with lower VL, while older age is associated with higher VL [12]. Other factors associated with VL include race, with some studies reporting lower VL in black compared with white groups [13,14].

The association between VL and CD4+ cell count, a marker of immune status, is well described. A study reported that an average of 1 log10 increase in viral load is associated with a 55 cell/mm3 CD4+ cell count decrease [15]. But Rodríguez et al. evaluated the association between VL on rate of CD4+ decline in untreated patients in two US cohorts and concluded that presenting VL predicts the rate of CD4+ cell decline only minimally [16]. It is likely that a mixture of factors drives CD4+ cell losses in HIV infection. It is therefore important to understand an individual’s risk for disease progression as this may have profound implications for therapeutic management decisions.

In view of the prognostic importance of VL and possible demographic differences in VL in ART-naive HIV-1 patients, it is important to conduct locally relevant studies to identify the risk factors for high viral loads. Such information could guide programmatic policies, especially with respect to ART initiation. The purpose of this study was to determine the HIV-1 VL levels among ART-naive patients and to identify the association between patients’ demographic, clinical and laboratory characteristics with VL.

Methods
This was a cross-sectional study in which 224 patients (≥15 years of age) accessing care at the Jos University Teaching Hospital (JUTH) AIDS Prevention Initiative in Nigeria (APIN) ART treatment centre from October 2010 to April 2011 were consecutively recruited. Patients were eligible for ART based on the Nigerian National Adult ART guidelines at time of enrolment [17], which closely followed the World Health Organization (WHO) guidelines at the time of patient enrolment [18]. Patients who were on ART prior to enrolment were excluded from the study. The study used data already captured in the electronic database as a part of ongoing HIV/AIDS care, treatment and support programmes. Data regarding: demographics (age, sex, marital
status, occupation, residence, education level, mode of HIV transmission, spouse HIV status, spouse ART status); clinical; World Health Organization (WHO) staging; pulmonary tuberculosis (PTB), oropharyngeal candidiasis and Kaposi’s sarcoma (KS), and laboratory parameters; hepatitis B virus (HBV) status; HIV-1 RNA viral load and CD4⁺ cell count were extracted. For HIV-1 serodiagnosis, collected whole blood samples and plasma were tested using rapid HIV tests: Uni-Gold (Trinity Biotech Plc, Bray, Ireland) and Determine HIV-1/2 test (Determine Alere Medical, Japan) with Statpak (Chembio Diagnostic Systems, New York, USA) as the tiebreaker assay for resolving discordant results. T-lymphocyte count (CD4⁺) was measured by Partec flow Cytometry (Munster, Germany). Hepatitis B surface antigen was determined using enzyme immunoassay (EIA) (Monolisa HBsAg Ultra3, Bio-Rad, Hercules, CA, USA). The plasma portion of the sample was separated after centrifugation and stored at -80°C for HIV-1 RNA viral load testing. The HIV-1 RNA viral load was measured using the Roche Cobas Amplicor HIV-1 Monitor (version 1.5, Roche Diagnostics GmbH, Mannheim, Germany). PTB was diagnosed from clinical symptoms (including chronic cough ≥2 weeks and weight loss), chest radiographs and a positive sputum smear microscopy using Ziehl–Neelsen (Z-N) staining for Mycobacterium tuberculosis (Mttx) acid-fast bacilli (AFB). Ethical clearance for the study was obtained from the JUTH institutional review board and the enrolled patients provided written informed consent. The use of secondary data was approved by the Harvard School of Public Health and JUTH APIN management.

Data analysis

To determine whether VL was related to demographic and clinical variables, we used a log-linear model in which we included demographic and clinical variables likely to correlate with natural log (log_e) transformed VL. We excluded individuals for whom there was missing information for any of the explanatory variables considered.

A model in which all variables were added including two-way interactions and gender, with all other explanatory variables was built. Using a backward stepwise procedure, the least significant of the variables was removed and the model tested again. This procedure was repeated until further removal of a variable did not improve the Akaike Information Criterion (AIC). The final statistical model accounted for 67% variability in the data (adjusted R² = 0.67). The R statistical software (Statistical Computing, Table 1. Clinical and demographic information collected from study subjects stratified by gender

| Variable                     | Subgroup       | Female N (%) | Male (%) | P*    |
|------------------------------|----------------|--------------|----------|-------|
| Age (years)                  | Median (IQR)   | 34 (28–41)   | 32 (27–38) | 36 (32–43) | <0.001 |
| Mode of HIV transmission     | Heterosexual   | 219 (97.7)   | 129 (97.7) | 90 (97.8) | 0.955  |
|                              | Transfusion    | 5 (2.2)      | 3 (2.3)   | 2 (2.2)  |        |
| Educational Level            | Illiterate     | 23 (10.2)    | 13 (9.9)  | 10 (10.9) | 0.982  |
|                              | Primary        | 49 (21.8)    | 30 (22.7) | 19 (20.7) |        |
|                              | Secondary      | 81 (36.1)    | 47 (35.6) | 34 (37)  |        |
|                              | Tertiary       | 71 (31.7)    | 42 (31.8) | 29 (31.5) |        |
| Marital status               | Divorced       | 3 (1.3)      | 2 (1.5)   | 1 (1.1)  | 0.118  |
|                              | Married        | 148 (66.0)   | 79 (59.9) | 69 (75)  |        |
|                              | Separated      | 12 (5.3)     | 9 (6.8)   | 3 (3.3)  |        |
|                              | Single         | 45 (20.0)    | 29 (22)   | 16 (17.4) |        |
|                              | Widowed        | 16 (7.1)     | 13 (9.9)  | 3 (3.3)  |        |
| Partner HIV status           | Negative       | 119 (53.1)   | 78 (59.1) | 41 (44.6) | 0.041  |
|                              | Positive       | 105 (46.8)   | 54 (40.9) | 51 (55.4) |        |
| Spouse ART                   | Negative       | 202 (90.1)   | 117 (88.6)| 85 (92.4) | 0.494  |
|                              | Positive       | 22 (9.8)     | 15 (11.4) | 7 (7.6)  |        |
| HBV                          | Negative       | 208 (92.8)   | 124 (93.9)| 84 (91.3) | 0.599  |
|                              | Positive       | 16 (7.1)     | 8 (6.1)   | 8 (8.7)  |        |
| PTB                          | Negative       | 198 (88.3)   | 120 (90.9)| 78 (84.8) | 0.204  |
|                              | Positive       | 26 (11.6)    | 12 (9.1)  | 14 (15.2) |        |
| Candidiasis                  | Negative       | 148 (66.0)   | 90 (68.2)| 58 (63)  | 0.474  |
|                              | Positive       | 76 (33.9)    | 42 (31.8)| 34 (37)  |        |
| Kaposi’s sarcoma             | No             | 216 (96.4)   | 127 (96.2)| 89 (96.7) | 0.985  |
|                              | Yes            | 8 (3.5)      | 5 (3.8)   | 3 (3.3)  |        |
| WHO staging                  | 1              | 41 (18.3)    | 25 (18.9)| 16 (17.4) | 0.16   |
|                              | 2              | 54 (24.1)    | 37 (28)  | 17 (18.5) |        |
|                              | 3              | 74 (33.0)    | 44 (33.3)| 30 (32.6) |        |
|                              | 4              | 55 (24.5)    | 26 (19.7)| 29 (31.5) |        |
| CD4⁺ cell count (cells/mm³)  | Median (IQR)   | 193.0 (92.7–284.0) | 202 (122.3–299.0) | 178 (69–269.8) | 0.075 |

* P values are based on chi-squared contingency table test, Fisher’s exact test for groups with less than five counts, and Mann–Whitney U-tests for age and CD4⁺ cell count.

N: number of subjects; IQR: interquartile range; ART: antiretroviral therapy; HBV: hepatitis B virus; PTB: pulmonary tuberculosis; WHO: World Health Organization.
Vienna, Austria) was used for the data analyses and P values of <0.05 were considered statistically significant.

Results
The characteristics of the study participants, summarised in Table 1, show that the participants had a median age of 34 years (interquartile range [IQR] 28–41) with females in the majority (59%). Heterosexual transmission was the predominant mode of HIV transmission (98%). Approximately 10% of the participants had no formal education, while the proportion of those with secondary education (36%) was higher in comparison to other levels of education. Most of the participants were married with a little above half reporting their partner HIV status as negative. Other infections such as HBV, candidiasis, and Kaposi’s sarcoma were uncommon with each having a prevalence of less than 10%, while the prevalence of PTB was above 10%. Over half of the participants were at WHO clinical stage 3 or 4. When the clinical and demographic characteristics of females and males were compared (Table 1), females had a lower median age than men (32 versus 36 years; P<0.001), while a higher proportion of males than females had HIV-positive partners (55% men compared to 41% females; P=0.041). There was no significant difference in other demographic and clinical characteristics between females and males. A summary of the mean (log transformed) viral load for the different categories is presented in Table 2. The results showed females compared to males and PTB negative compared to PTB positive patients had a significantly higher VL (14.9 log, versus 11.5 log, P=0.003 and 11.31 log, versus 11.89 log., P=0.047, respectively). The difference (β) in the mean change in VL between males and females was log 0.64 copies/mL (Table 2). The change in VL between males and females is illustrated in Figure 1. Viral load tended to decrease with increasing CD4+ cell counts in females, but remained relatively unchanged in males across all values of CD4+ cell counts (Figure 1).

Discussion
Our result showed that VL in ART-naive HIV-1-infected patients was significantly influenced by sex, PTB status and CD4+ cell count. Females and patients with PTB had a significantly higher VL, while viral load decreased with increasing CD4+ cell count; however, this decrease was more significant in females compared to males.

HIV RNA levels were significantly higher at lower CD4+ cell counts in females than males in this study, whereas earlier studies [8,19–21] found HIV VL to be lower in females compared to males at similar stages of HIV

| Parameter | Log transformed mean viral load (CI) | β (CI)† | P |
|-----------|------------------------------------|--------|----|
| Sex       |                                    |        |    |
| Female    | 14.9 (11.71–16.94)                 | 3.38 (1.13–5.64) | 0.003 |
| Male*     | 11.5 (8.14–13.718)                 | 0      |    |
| Mode of transmission |                                    |        |    |
| Heterosexual | 11.54 (11.67–16.99)               | –0.12 (–1.39–1.15) | 0.858 |
| Transfusion  | 11.65 (11.37–17.52)              | 0      |    |
| Education level |                                    |        |    |
| Illiterate  | 11.42 (11.71–16.94)               | –0.13 (–0.83–0.57) | 0.719 |
| Primary     | 11.73 (12.07–17.21)               | 0.18 (–0.37–0.73) | 0.516 |
| Secondary   | 11.69 (12.04–17.15)               | 0.14 (–0.33–0.61) | 0.561 |
| Tertiary*   | 11.55 (11.97–16.96)               | 0      |    |
| Marital status |                                    |        |    |
| Divorced    | 12.18 (11.71–16.94)               | 0.87 (–0.9–2.65) | 0.333 |
| Married     | 11.15 (11.2–15.39)                | –0.15 (–0.91–0.59) | 0.686 |
| Separated   | 11.87 (11.71–16.29)               | 0.56 (–0.54–1.67) | 0.316 |
| Single      | 11.46 (11.53–15.69)               | 0.16 (–0.73–1.04) | 0.728 |
| widowed*    | 11.31 (11.29–15.62)               | 0      |    |
| Partner HIV status |                                    |        |    |
| Negative    | 11.52 (11.71–16.94)               | –0.16 (–0.61–0.29) | 0.476 |
| Positive*   | 11.68 (11.89–17.08)               | 0      |    |
| Spouse on ART |                                    |        |    |
| No          | 11.7 (11.71;16.94)                | 0.21 (–0.49–0.91) | 0.564 |
| Yes*        | 11.49 (11.46;16.75)               | 0      |    |
| HBV         |                                    |        |    |
| Negative    | 11.34 (11.71–16.94)               | –0.52 (–1.26–0.22) | 0.169 |
| Positive*   | 11.86 (12.13–17.56)               | 0      |    |
| PTB         |                                    |        |    |
| Negative    | 11.31 (11.71–16.94)               | –0.98 (–1.24–0.08) | 0.047 |
| Positive*   | 11.89 (12.17–17.65)               | 0      |    |
| Candidiasis |                                    |        |    |
| Negative    | 11.61 (11.71–16.94)               | 0.027 (–0.44–0.49) | 0.911 |
| Positive*   | 11.58 (11.68–16.93)               | 0      |    |
| Kaposi’s sarcoma |                                    |        |    |
| No          | 11.6 (11.67–16.94)                | 0.06 (–0.99–1.12) | 0.910 |
| Yes*        | 11.6 (11.38–17.1)                 | 0      |    |
| WHO staging |                                    |        |    |
| 1           | 11.3 (11.67–16.94)                | –0.31 (–1.09–0.47) | 0.432 |
| 2           | 11.6 (12.05–17.21)                | 0.01 (–0.69–0.72) | 0.977 |
| 3           | 11.8 (12.23–17.36)                | 0.18 (–0.42–0.77) | 0.555 |
| 4*          | 11.627 (12.14–17.1)               | 0      |    |
| Log CD4 cell count |                                    |        |    |
| 0*          | –0.04 (–0.39–0.31)                | 0.008  |    |
| Age         | 0.01 (–0.02–0.03)                 | 0.636  |    |
| Sex log CD4 cell count* |                                    |        |    |
| Females log CD4 cell count* | –0.64 (–1.07–0.19) | 0.005 |
| Males log CD4 cell count* | 0 |    |    |

* Reference group for factor variables.
† β: difference of means for group variables and the slope for continuous variables.
ART: antiretroviral therapy; HBV: hepatitis B virus; PTB: pulmonary tuberculosis; WHO: World Health Organization.
A comprehensive immunological assessment that included the underpinning sex differences in HIV disease progression is unclear. To AIDS is faster in women than in men [19,23]. The mechanism studies showed that at the same level of viraemia, progression in our setting [22]. Consistent with the finding of this study, some present for treatment at more advanced stage of HIV disease in our setting [22]. Consistent with the finding of this study, some studies showed that at the same level of viraemia, progression to AIDS is faster in women than in men [19,23]. The mechanism underpinning sex differences in HIV disease progression is unclear. A comprehensive immunological assessment that included the analysis of CD4+ and CD8+ T-cell activation of 980 ART-naive HIV-1-infected subjects (514 men and 109 women) followed at US sites, showed a substantial sex difference in the response of plasmacytoid dendritic cells (pDCs) to HIV-1. Plasmacytoid dendritic cells derived from women produce markedly more interferon-alpha (IFN-alpha) in response to HIV-1-encoded Toll-like receptor 7 (TLR7) ligands than pDCs derived from men, resulting in stronger secondary activation of CD8+ T cells. The sex difference in TLR-mediated activation of pDCs may account for higher immune activation in women compared to men at a given HIV-1 viral load and provide a mechanism by which the same level of viral replication might result in faster HIV-1 disease progression in women compared to men [23]. In line with the WHO HIV treatment approach [24], which recommends ART in all adults living with HIV regardless of WHO clinical stage and CD4 cell count, early identification of HIV-infected females and initiation of treatment will improve treatment prognosis and programmatic success. Unlike previous studies where older patients tended to have higher VL levels [12], this study did not find any significant association between age and HIV VL in ART-naive patients.

Consistent with other studies [25,26], we found a significantly higher VL in HIV/PTB co-infected patients compared to those infected with HIV only. Available evidence suggests that the increase in HIV viraemia that occurs in association with tuberculosis may result from upregulation of CXCR4 and CCR5 on CD4+ T cells, thereby causing acceleration of HIV infection [27]. Other cellular mechanisms involve induction by Mtb of HIV-1 replication in HIV-infected cell lines, including in mononuclear cells from HIV-1 infected subjects [28]. When mononuclear cells from subjects with tuberculosis (TB) are activated, they become more susceptible to a productive infection by HIV-1 [29,30]. Additionally, the cytokine profile of the tuberculous microenvironment is conducive for HIV-1 replication [31]. Considering the effect of TB on viral load, and the high burden of TB in Nigeria [32], TB chemoprophylaxis in HIV-1-infected patients in our setting might be beneficial and should be explored. The cross-sectional design of this study has some inherent limitations that should be considered in interpreting the results of the study. HIV RNA was measured at a single point in time and hence we are unable to describe the trend in disease progression. Furthermore, the interval between seroconversion was not considered in evaluating the association between the VL. However, using the population baseline VL we were able to describe the association between several demographic and clinical characteristics and VL in ART-naive patients.

Conclusion

In ART-naive HIV-1-infected patients in our setting, females compared to males had significantly higher VL and lower CD4+ cell counts at the same VL threshold. This data suggests that more females than males were presenting late for treatment in our setting and hence were more likely to be at a higher risk of rapid progression to AIDS. Therefore, gender-based strategies for early identification and integration of females into care are required in this setting to mitigate against rapid progression to AIDS. Further studies in African settings are required to characterise the pattern and magnitude of viral load that may be unique to males and females in this setting.

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