Laboratory Markers and Characteristics of New HIV Infections Among Adolescents in Zambia

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Research article

Keywords: HIV RNA, New HIV Infections, Adolescents, Young People, CD4+ T-cell Count, Viral Load, Hepatitis B, Syphilis

DOI: https://doi.org/10.21203/rs.3.rs-42500/v1

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Abstract

Background

We aimed to explore HIV RNA (ribonucleic acid) virologic levels greater than 1,000 copies/millilitre (ml), among HIV-positive adolescents aged 15–24 years, establish the spread of CD4 T-cell counts and inspect characteristics of adolescents presenting with new HIV infections, including co-infections with Hepatitis B virus and syphilis.

Methods

We analysed data from the Zambia Population-based HIV Impact Assessment 2016 survey. Two-stage stratified cluster probability sample design was used to select the target population. Our study truncated the population to focus on the age-group 15–24 whose biomarker tests and household information were complete. Our primary outcome measure was "New HIV-positive Infections among 15-24-year-olds" defined as HIV-positive biomarker samples presenting with HIV RNA $\geq$ 1,000 copies/ml without detectable ARVs. We tested associations between new HIV infections and clinical characteristics using negative binomial models adjusting for age, sex, education, marital-status, residence among several covariates.

Results

Overall, 2.3% ([166/7320], 95% CI: 1.9–2.6) adolescents aged 15–24 years were diagnosed with new HIV infections, with greater proportions among females (3.3% [139/4,165], 95% CI: 2.8–3.9) than males (0.86% [27/3,155], 95% CI: 0.6–1.2). Almost half (47.6%) of seroconversions had HIV RNA $\geq$ 50,000 copies/ml and an average CD4 + T-cell count of 479 cells/mm $^3$. HBV– positive adolescents (IRR 8.6, 95% CI: 2.9–24.9, P < 0.001, model 2) were at increased risk of new infections unlike those testing positive for syphilis antibodies (IRR 1.22, 95% CI: 0.2–8.3, P < 0.84). Adjusting for confounders revealed that being married or cohabiting, testing positive for HBV, being a rural resident and attaining higher than secondary education emerged as strongest correlates of new infections.

Conclusion

High baseline levels of viral load and low CD4 + T-cell count in recent HIV infections among adolescents indicate weak immune repertoire at first diagnosis, increasing the risk of contagion. As the epidemic continues to spread within the adolescent population, HIV-infection will become more complex and greater proportions of adolescents will likely be infected by regular partners. This suggests growing need for interventions targeted at stable partnerships and intensified public health campaigns specific for adolescents.

Background

The human immunodeficiency virus (HIV) infection is one common cause of adolescent hospitalization because of the continuum of opportunistic infections and high burden of protracted complications due to HIV/AIDS (1). UNAIDS reveals that in 2015, 260 000 [180 000–340 000] new HIV infections were diagnosed among adolescents in sub-Saharan Africa, estimated at 29 adolescents acquiring HIV every hour (2). Estimates further indicate that young girls aged 15–19 accounted for almost 80% of these recent infections (2). The proportion of young people living with HIV rose globally by 30% between 2005 and 2016 and those dying due to AIDS-related illnesses tripled, making it the only age group to have experienced an increase (3). Adolescents’ risk of acquiring HIV is closely correlated, among other factors, with age at sexual debut, low condom use, low counseling and testing coverage, legal and structural barriers (2, 4).

Irrespective of achievements so far made in responding to HIV, approximately 60,000 persons were diagnosed with new HIV infections in Zambia in 2015 comprising 50,000 adults and 8,900 children (2, 5). Youth are the least likely than any other age group to be aware of their infection. For example, only 42% of adolescents (aged 15–24) in Zambia knew their HIV status compared to 67% among adults 15–59 years in 2016 (6). Also, population-based survey data from sub-Saharan Africa shows that only 9% of young men 15–19 compared to 13% of their girls contemporaries had tested and received results for HIV in the last 12 months (7, 8). Compounding the status quo are sex disproportions in the prevalence of HIV. Within the population aged 20–24, females exhibit higher prevalence levels than males (5.6% versus 1.8%) (5). Adolescent girls who reported being divorced, separated, or widowed had higher (13.4%) HIV prevalence than their currently married and never-married counterparts (6.2% and 4.8% respectively) (9). In spite of growing accessibility to effective HIV prevention tools, methods and substantial scale-up of HIV treatment, attainment of viral load suppression (VLS) remains distinctly low in the younger population: 34% in HIV-positive females and 35.7% among HIV-positive males aged 15 to 24 compared to 73.5% in HIV-positive women and 73% in HIV-positive men aged 45 to 59 years (10). This rate of progress towards slowing the incidence of HIV acquisition, bolstering accessibility to treatment, and stopping AIDS-related deaths particularly among adolescents demands expansion and scaled up in order to reach high-incidence locations and maximize impact (11).
Because several clinical studies of HIV infection (5, 12–14), often disaggregate the population aged 0–14 years as children and 15–49 years as adults, in so doing, the probability of missing distinctive features of recent infections among a special group of adolescents aged 15–24 years is high. The age range 15–24 years aligns with the concept that adolescence might best be considered as ranging from 10 to 24 years because the transition period from childhood to adulthood continues into the twenties (15), and occupies a substantive part of their life course and shifting patterns of health and wellbeing (16). For this reason, it has been proposed that when adolescence is perceived as the population of young people from 10 to 24 years (15), it would help create opportunities for adolescents to acquire valuable assets and capabilities relevant, among others benefits, to averting health risk (17). However, for this study, we consider young people aged 15–24 years as adolescents. Herein, we explore the HIV RNA (ribonucleic acid) virologic levels greater than 1,000 copies/millilitre (ml) with undetectable antiretrovirals (ARVs), among HIV-positive adolescents aged 15–24 years, determine the distribution of CD4 + T-cell counts and inspect characteristics of adolescents presenting with new HIV infections, including co-infections with Hepatitis B virus (HBV) and syphilis.

Methods

Study Design and Sample

The Zambia Population-based HIV Impact Assessment (ZAMPHIA) 2016 dataset, on which this study is based, was a cross-sectional survey, nationally representative as described in the ZAMPHIA 2016 report (5), the study utilized the two-stage stratified cluster sample method, a probability proportional to size and an equal probability method to select the target population. The total sampled households were 13,441, comprising 5,205 eligible females and 4,337 eligible males aged 15–24. Among the eligible population aged 15–24 years, 88.1% (4,585/5,205) of women and 80.5% (3,491/4,337) of men completed the interview. The response rate for biomarker testing was 90.6% (4,716/5,205) for females and 90.0% (3,903/4,337) for males. Therefore, the sample size for our study was 7,320 comprising 4,165 girls and 3,155 boys aged 15–24 who undertook biomarker testing in the ZAMPHIA 2016 study.

Field-based Biomarker Testing

All results for field-based tests were provided to clients and those testing positive were referred for treatment. As detailed in the ZAMPHIA 2016 report (5), the following tests were conducted in the field.

HIV Home-Based Testing and Counselling (HBTC) – This was performed on all sampled households. First, a screening test using Determine™ HIV-1/2 (Abbott Molecular Inc., Des Plaines, Illinois, United States) was performed. Participants diagnosed with a non-reactive result were reported HIV-negative. A confirmatory test using Uni-Gold™ (Trinity Biotech, plc. Wicklow, Ireland) was conducted for participants with reactive test results (18).

Hepatitis B Testing – As with HBTC, testing for HBV was done in every sampled household on individuals of all ages. The serological hepatitis B surface antigen rapid diagnostic test, Determine™ HBeAg, was used to determine acute or chronic HBV infection (5).

Syphilis Testing – All sampled participants in the age range 15–59 were tested for syphilis on DPP Syphilis Screen and Confirm Assay (Chembio, Medford, NY) in order to concurrently detect antibodies against non-Treponemal and Treponema pallidum antigens. Further confirmatory test was done on SD BIOLINE Syphilis 3.0 (Abbott Molecular Inc., Chicago, Illinois, United States) (5).

Laboratory-based Biomarker Tests

The following tests were performed in reference laboratories in Lusaka and Ndola.

Viral Load (VL) Testing – This test, also known as the HIV-1 RNA, was done for only verified positives using the Roche COBAS AmpliPrep Instrument and COBAS TaqMan 48 Analyzer on COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (Roche Molecular Diagnostics, Branchburg, New Jersey, United States) (5). For samples with insufficient volume of plasma, HIV-1VL was measured from dried blood spot with the use of Abbott RealTime HIV-1 Assay (Abbott Molecular, Wiesbaden, Germany) (5). Definite HIV-positive samples also received a CD4 + T-cell count test on the Pima™ CD4 Analyzer (Abbott Molecular Inc., Chicago, Illinois, United States) (5). Additionally, 5.0% of randomly selected HIV-negative samples underwent the CD4 + T-cell count test.

Estimating New HIV Infections

To facilitate estimation of new HIV infections, the ZAMPHIA made use of two laboratory-based testing algorithms. 1) HIV-1 LAg Avidity enzyme immunoassay (EIA) (Serodia Biosciences Corporation, Portland, Oregon, United States) and VL (see Fig. 1) HIV-1 LAg Avidity EIA, VL, and ARV detection (5). Samples for which the median normalized optical density (ODn) was ≤ 1.5 were categorized as newly infected specimens precipitating VL testing. Following this investigation, outcomes of VL < 1,000 copies/ml were labelled as old infections and VL ≥ 1,000 copies/ml – new infections (5). When the ARV-controlled algorithm was considered, samples with VL ≥ 1,000 copies/ml in which the presence of ARVs was
detected were also grouped as old infections. Conversely, samples for which VL ≥ 1,000 copies/ml with absence or undetectable ARVs were then categorized as new infections (5, 19).

Figure 1. STARHS biomarker detection schema used in estimating new HIV infections

[Figure 1 Here]

Reproduced from FHI (20).

The serological testing algorithm for recent HIV seroconversion (STARHS) estimates the immunological response against the virus on the basis of certain HIV antibody concentration, proportion (BED), isotype or avidity (20, 21). The time lapse from the point of acquisition (when antibodies can be detected) to the cut-off value defining confirmed infection status, or the window period, should be definitively established which is fundamental to the STARHS assays’ capacity to specify the rate of incidence for a given population (20, 22).

**Measures**

The study aimed to find evidence to answer the following questions; Among the population aged 15–24 years, what proportion of HIV-positive samples indicated HIV RNA ≥ 1,000 copies/ml with undetectable ARVs? What proportion of HIV-positive specimens with HIV RNA ≥ 1,000 copies/ml without detectable ARVs, tested positive for HBV and syphilis antibody test? What is the CD4 + T-cell count distribution of HIV-positive samples with HIV RNA ≥ 1,000 copies/ml without detectable ARVs, for the population 15–24 years? The CD4 + T-Cell count profiles how the immune system is functioning. The higher the T-Cell count, the better. As HIV infection progresses, the number of T-cells falls. The standard range for CD4 + T-cells runs between 500 and 1,500 cells/mm³ (Pantaleo & Fauci, 2005). However, most opportunistic infections (OIs) are found among patients with CD4 counts < 200 cells/mm³ (23). To investigate other determinants of new HIV infection in adolescents, our study endeavoured to answer the following questions. What underlying factors are associated with new HIV infections in adolescents. (e.g. new HIV infections with HBV and/or active syphilis antibodies)? Are there differences in new infections in adolescents depending on their background characteristics? A priori, we expected associations between new HIV infections in the population and the predictor variables. Our primary outcome measure (recent HIV infections in the population 15–24) was therefore, regressed on the four main predictor variables (age, sex, positive syphilis test and positive HBV test) across all models.

**Analytic Variables**

**Outcome**

The outcome variable was “New HIV-positive Infections among 15-24-year-olds” defined as HIV-positive biomarker samples with HIV RNA ≥ 1,000 copies/ml without detectable ARVs”. It was a count variable. The focus for analysis was on samples that tested positive with HIV RNA ≥ 1,000 copies/ml with undetectable ARVs as these samples were classified as new infections. HIV-1 RNA is the response marker for antiretroviral therapy (ART). HIV RNA tests measure the amount of HIV in the blood. A low RNA means a person is less likely to transmit HIV. A patient’s pre-ART RNA level and the extent of RNA reduction following commencement of ART offers predictive insight about the prospects of disease progression (24).

**Predictor**

Predictor variables were clustered into laboratory markers (CD4 + T-cell count distribution, testing positive for hepatitis B virus and syphilis antibody test) and; demographic parameters (age and sex). The two laboratory markers, presence of hepatitis B virus and syphilis, were appropriate for assessing co-infection in HIV-positive individuals. HIV-positive persons who are concurrently positive for hepatitis B ‘e’-antigen (HBeAg) usually present with higher hepatitis B viral load and fail to respond to antiviral treatment as positively as those with HBeAg-negative hepatitis B (25). Similarly, syphilis demonstrates an adverse impact on HIV infection, often revealing increases in RNA and corresponding decreases in CD4 + T-cell counts during active syphilis infection. Thus, individuals with HIV-syphilis co-infection are at increased risk of neurological complications and treatment failure (26).

**Covariates**

The third set of variables we considered were socioeconomic covariates deemed as influential determinants of new infections. They included marital status, education [highest level achieved], residence [rural, urban], wealth index, HIV testing history, and awareness of one’s HIV-positive status.

**Statistical Analysis**

We summarized incidence of new HIV infections and immunological profiles according to demographic and socioeconomic characteristics. The likelihood ratio chi-square χ² test aimed at testing whether the model containing the full set of predictors fits significantly better than a null (intercept only) model.; differences between predictor variables were assessed using Pearson chi-square χ² test of independence or Fisher’s exact
test for variables with an expected frequency of cells of five or less (e.g. education). Continuous predictors were analyzed with a Student’s t test for normally distributed variables, and the Wilcoxon-Mann-Whitney test (for age) or the Kruskal–Wallis test (for wealth quintile) for variables that did not have normal distributions. Univariate and bivariate analysis of variables reports proportions and binomial exact output at 95% confidence intervals (CI).

The number of new HIV infections is a count variable and closely follows a poisson distribution. We modelled the number of recent infections among young people using negative binomial regression. The negative binomial relative to count models such as the poisson or zero-inflated models, was considered suitable because the outcome variable was over-dispersed and did not have so many zeros. We further used generalized estimating equations and robust standard errors to determine factors independently and mutually associated with new HIV infections. When exponentiated, negative binomial regression coefficients provide the ratio of expected count per unit increase in exposure, referred to as the incident rate ratio (IRR) (27). We started by examining independent associations of each predictor variable and covariate (one at a time) with the outcome variable - new HIV infections (model 1). In model 2, all predictors and covariates with a P-value of up to 0.2 in model 1 were included. Associations were adjusted for potential confounders: education, marital status, wealth quintile, awareness of HIV-positive status, residence (rural/urban), number of sex partners in past 12 months and history of HIV testing. Model 3 considered all variables that were statistically significant at \( p \leq 0.05 \) in model 2. Probabilities for removal and entry of predictor variables into the models were set at p-values of 0.20. IRRs at 95% confidence intervals are reported, and p-values less than 0.05 were considered statistically significant. All statistical analysis was done in STATA 14.2 software (28).

**Results**

Overall incidence of new HIV infections for adolescents was from the general survey 2.3% ([166/7,320], 95% CI: 1.94–2.63). Infections were notably greater in females (3.34%, 95% CI: 2.81–3.90) compared to male adolescents (0.86%, 95% CI: 0.56–1.24).

**Characteristics of Adolescents Testing Newly Positive for HIV**

Table 1 below shows detailed characteristics of adolescents testing newly positive to HIV. More females (83.7%, 95% CI: 77.2–89.0) than males (16.3%, 95% CI: 11.0–22.8) and more older adolescents (66.3%, 95% CI: 58.5–73.4) aged 20–24 compared to younger ones (33.7%, 95% CI: 26.6–41.5) aged 15–19 were diagnosed with recent HIV infections. Socioeconomic characteristics indicate larger proportions of unmarried adolescents (63.9%, 95% CI: 56.0–71.2) than married contemporaries (36.1%, 95% CI: 28.8–43.9) testing newly positive for HIV. New infections were observed to rise with wealth and education level. For instance, 28.0% (95% CI: 21.3–35.6) of participants in the highest wealth quintile compared to 7.3% (95% CI: 0.56–1.24) from the lowest tested positive. While 31.9% adolescents with primary education relative to 63.9% with secondary education had seroconverted. Behavioral attributes further show that two-thirds (67.6%) of adolescents that reported having had one sexual partner in 12 months prior to the survey compared to 19.9% with no partner had acquired the virus. Of all newly infected adolescents, only 14.5% were aware of their positive status, a consequence of low levels of HIV testing among young people (29–31).
Table 1
Characteristics of Adolescents Testing Newly Positive for HIV

| Predictor                           | Total (n = 166) | Percentage of New HIV Infections | 95% CI       |
|-------------------------------------|-----------------|---------------------------------|--------------|
| **Sex**                             |                 |                                 |              |
| Male                                | 27              | 16.3 (27/166)                   | 11.0–22.8    |
| Female                              | 139             | 83.7 (139/166)                  | 77.2–89.0    |
| **Age**                             |                 |                                 |              |
| 15–19                               | 56              | 33.7 (56/166)                   | 26.6–41.5    |
| 20–24                               | 110             | 66.3 (110/166)                  | 58.5–73.4    |
| **Residence**                       |                 |                                 |              |
| Rural                               | 70              | 42.2 (70/166)                   | 34.6–50.0    |
| Urban                               | 96              | 57.8 (96/166)                   | 49.9–65.4    |
| **Marital status**                  |                 |                                 |              |
| Not married                         | 106             | 63.9 (106/166)                  | 56.0–71.2    |
| Married                             | 60              | 36.1 (60/166)                   | 28.8–43.9    |
| **Wealth quintile**                 |                 |                                 |              |
| Lowest                              | 12              | 7.3 (12/164)*                   | 3.8–12.4     |
| Second                              | 22              | 13.4 (22/164)                   | 8.6–19.6     |
| Middle                              | 36              | 22.0 (36/164)                   | 15.9–29.1    |
| Fourth                              | 48              | 29.3 (48/164)                   | 22.4–36.9    |
| Highest                             | 46              | 28.0 (46/164)                   | 21.3–35.6    |
| **Education level**                 |                 |                                 |              |
| No education                        | 2               | 1.2 (2/166)                     | 0.15–4.3     |
| Primary                             | 53              | 31.9 (53/166)                   | 24.9–39.6    |
| Secondary                           | 106             | 63.9 (106/166)                  | 56.0–71.2    |
| Tertiary                            | 5               | 5.0 (5/166)                     | 0.9–6.8      |
| **Active syphilis**                 |                 |                                 |              |
| Positive                            | 12              | 7.2 (12/166)                    | 3.8–12.3     |
| Negative                            | 154             | 92.8 (154/166)                  | 87.7–96.2    |
| **Hepatitis B virus**               |                 |                                 |              |
| Positive                            | 11              | 6.6 (11/166)                    | 3.3–11.5     |
| Negative                            | 155             | 93.4 (155/166)                  | 88.4–96.6    |
| **Number of sex partners in last 12 months** |     |                                 |              |
| No partner                          | 30              | 19.9 (30/151)**                 | 13.8–27.1    |
| One partner                         | 102             | 67.6 (102/151)                  | 59.5–74.9    |
| More than one                       | 19              | 12.6 (19/151)                   | 7.7–18.9     |
| **Ever tested for HIV**             |                 |                                 |              |
| Yes                                 | 132             | 79.5 (131/166)                  | 71.9–84.5    |

*“Wealth quintile” had 164 total observations instead of 166 because of missing values

**“Number of sex partners in last 12 months” also had less observations (151) than the expected 166 due to missing values
| Predictor                  | Total (n = 166) | Percentage of New HIV Infections | 95% CI   |
|----------------------------|-----------------|---------------------------------|----------|
| No                         | 34              | 20.5 (34/166)                   | 15.1–28.0|
| Aware of HIV + status      |                 |                                 |          |
| Aware                      | 24              | 14.5 (24/166)                   | 9.5–20.7 |
| Not aware                  | 142             | 85.5 (142/166)                  | 79.2–90.5|
| * "Wealth quintile* had 164 total observations instead of 166 because of missing values |
| ** "Number of sex partners in last 12 months" also had less observations (151) than the expected 166 due to missing values |

Virologic Outcomes of Adolescent with New HIV Infections

Slightly more than one third of new infections (36.7% [61/166], 95% CI: 29.41–44.57) had HIV RNA viral loads clustered in the 10,001–50,000 copies/ml viral load category (see Fig. 2a.). HIV RNA viral load above 50,000 copies/ml is strongly associated with the risk of transmission (32). Findings show that the mean HIV RNA viral load for new HIV infections in adolescents was 164,183 copies/ml (95% CI: 64,886 – 263,480 copies/ml, Fig. 2b.), more than three times the 50,000 copies/ml threshold. Cumulatively, nearly half (47.6% [79/166], 95% CI: 39.80–55.47) of newly infected adolescents were diagnosed with HIV RNA viral load above 50,000 copies/ml (see red reference line in Fig. 2b.). Disproportionately high viral load levels beyond 500,000 copies/ml were detected only in younger females adolescents aged 15–19 (1.81% [3/166]), (Fig. 2c. and Fig. 2d.) suggesting a possibility of acute infection at time of first diagnosis. Dissimilar to the HIV incidence pattern, males had higher proportions (59.3% [16/27], 95% CI: 38.8–77.6) of HIV RNA ≥ 50,000 copies/ml than females (45.3% [63/139], 95% CI: 36.9–54.0). There was also an observed predisposition for slightly more (48.2% [27/56], 95% CI: 34.6–62.0, Fig. 2d.) younger adolescents (15–19) to have had viral loads greater than 50,000 copies/ml relative to the older cohort 20–24 years (45.5%, [50/110], 95% CI: 35.9–55.3).

Figure 2: Viral Load and CD4 T-Cell Count for New HIV Infection in Adolescents

[Figure 2 Here]

Figure 2a:

Figure 2b:

*Red reference line (yline) indicates the cut-off at HIV RNA 50,000 copies/ml

Figure 2c:

Figure 2d:

Immunological Status among New HIV Seroconverts

Study results further show an average CD4 + T-cell count of 479 cells/mm3 (95% CI: 445–513.6, Figs. 2b., 2c., and 3.) among newly HIV infected persons. Close to two-thirds (62% [103/166], 95% CI 54.20–69.46) had CD4 + T-cell count ≤ 500 cells/mm3 signaling poor immune repertoire (see Fig. 3. and Table 2.). There was a marginal variation in proportions by sex among individuals presenting with CD4 + T-cell count ≤ 500 cells/mm3. Specifically, 63.0% (17/27, 95% CI: 42.4–80.6) males virtually at par with females at 61.9% (86/139, 95% CI: 53.3–70.0), see Table 2.

Figure 3. CD4 T-Cell Count of Newly Infected Adolescents

[Figure 3 Here]

* Red reference line (xline) shows CD4 + T-cell count cut-off at 500 cells/mm³. The larger area on the left of the reference line reveals a greater proportion of adolescents presenting with decreased immunity at first diagnosis of a positive HIV serostatus.

On the other hand, age differentials revealed larger proportions (64.5% [71/110], 95% CI: 54.9–73.4) of older adolescents ages 20–24 years exhibiting diminishing immunological profiles of CD4 T-cell counts ≤ 500 cells/mm³ than younger adolescents 15–19 years (57.1% [32/56], 95% CI 43.2–70.3). Analysis to further understand the immunity status among seroconverts revealed that 29.5% (7/139 + 34/139, Table 2) females and 37% (7/27 + 3/27,) males had CD4 + T-cell count ≤ 350 cells/mm³. In addition, slightly more females (38.1%, 95% CI: 30.0–46.7) presented with stronger immunity at CD4 + T-cell count greater than 500 cells/mm³ compared to males (37.0%, 95% CI: 19.4–57.6) males. Table 2 below illustrates further details of immunological statuses of adolescents by different characteristics.
Table 2
Percentages of CD4+ T-Cell Count Levels for all Predictor Variables

| Predictor                  | ≤ 200 cells/mm³ | 201–350 cells/mm³ | 351–500 cells/mm³ | > 500 cells/mm³ |
|----------------------------|-----------------|-------------------|-------------------|-----------------|
| **Total**                  | 6.0 (10/166)    | 24.7 (41/166)     | 31.3 (52/166)     | 38.0 (63/166)   |
| **Sex**                    |                 |                   |                   |                 |
| Male                       | 11.2 (3/27)     | 25.9 (7/27)       | 25.9 (7/27)       | 37.0 (10/27)    |
| Female                     | 5.0 (7/139)     | 24.5 (34/139)     | 32.4 (45/139)     | 38.1 (53/139)   |
| **Age**                    |                 |                   |                   |                 |
| 15–19                      | 7.1 (4/56)      | 19.6 (11/56)      | 30.4 (17/56)      | 42.9 (24/56)    |
| 20–24                      | 5.5 (6/110)     | 27.3 (30/110)     | 31.8 (35/110)     | 35.5 (39/110)   |
| **Residence**              |                 |                   |                   |                 |
| Rural                      | 5.7 (4/70)      | 25.7 (18/70)      | 31.4 (22/70)      | 37.1 (26/70)    |
| Urban                      | 6.3 (6/96)      | 24.0 (23/96)      | 31.3 (30/96)      | 38.5 (37/96)    |
| **Marital status**         |                 |                   |                   |                 |
| Not married                | 6.6 (7/106)     | 18.9 (20/106)     | 36.8 (39/106)     | 37.7 (40/106)   |
| Married                    | 5.0 (3/60)      | 35.0 (21/60)      | 21.7 (13/60)      | 38.3 (23/60)    |
| **Wealth quintile**        |                 |                   |                   |                 |
| Lowest                     | 8.3 (1/12)      | 33.3 (4/12)       | 8.3 (1/12)        | 50.0 (6/12)     |
| Second                     | 4.5 (1/22)      | 13.6 (3/22)       | 50.0 (11/22)      | 31.8 (7/22)     |
| Middle                     | 2.8 (1/36)      | 25.0 (9/36)       | 33.3 (12/36)      | 38.9 (14/36)    |
| Fourth                     | 6.3 (3/48)      | 35.4 (17/48)      | 22.9 (11/48)      | 35.4 (17/48)    |
| Highest                    | 8.7 (4/46)      | 17.4 (8/46)       | 37.0 (17/46)      | 37.0 (17/46)    |
| **Education level**        |                 |                   |                   |                 |
| No education               | 0.0 (0/2)       | 50.0 (1/2)        | 50.0 (1/2)        | 0.0 (0/2)       |
| Primary                    | 1.9 (1/53)      | 26.4 (14/53)      | 24.5 (13/53)      | 47.2 (25/53)    |
| Secondary                  | 8.5 (9/106)     | 23.6 (25/106)     | 34.0 (36/106)     | 34.0 (36/106)   |
| Tertiary                   | 0.0 (0/5)       | 20.0 (1/5)        | 40.0 (2/5)        | 40.0 (2/5)      |
| **Active Syphilis**        |                 |                   |                   |                 |
| Positive                   | 0.0 (0/12)      | 25.0 (3/12)       | 33.3 (4/12)       | 41.7 (5/12)     |
| Negative                   | 6.6 (10/154)    | 24.7 (38/154)     | 31.2 (48/154)     | 37.7 (58/154)   |
| **Hepatitis B Virus**      |                 |                   |                   |                 |
| Positive                   | 0.0 (0/11)      | 18.2 (2/11)       | 36.4 (4/11)       | 45.5 (5/11)     |
| Negative                   | 6.5 (10/155)    | 25.2 (39/155)     | 31.0 (48/155)     | 37.4 (58/155)   |
| **Number of sex partners in last 12 months** | | | | |
| No partner                 | 3.3 (1/30)      | 20.0 (6/30)       | 33.3 (10/30)      | 43.3 (13/30)    |
| One partner                | 5.9 (6/102)     | 29.4 (30/102)     | 31.4 (32/102)     | 33.3 (34/102)   |
| More than one              | 5.3 (1/19)      | 15.8 (3/19)       | 42.1 (8/19)       | 36.8 (7/19)     |

**Ever tested for HIV**

Co-Infection in New HIV-positives
| Predictor | \(\leq 200\) cells/mm\(^3\) | 201–350 cells/mm\(^3\) | 351–500 cells/mm\(^3\) | > 500 cells/mm\(^3\) |
|-----------|-----------------|-----------------|-----------------|-----------------|
| Yes       | 5.3 (7/132)     | 27.3 (36/132)   | 31.8 (42/132)   | 35.6 (47/132)   |
| No        | 8.8 (3/34)      | 14.7 (5/34)     | 29.4 (10/34)    | 47.1 (16/34)    |

**Aware of HIV+ status**

|          | Aware          | Not aware      |
|----------|----------------|----------------|
|         | 12.5 (3/24)    | 4.9 (7/142)    |
| Aware   | 20.8 (5/24)    | 25.4 (36/142)  |
| Not aware | 33.3 (8/24)   | 31.0 (44/142)  |
|          | 33.3 (8/24)    | 38.7 (55/142)  |

The study considered two common co-infections in HIV-positive adolescents; syphilis and HBV. Granted that syphilis, HBV and HIV can be acquired in similar ways, notwithstanding that syphilis and HBV are more infectious, co-infection occurs frequently (25, 26). Among adolescents with recent HIV infections, 7·2% (12/166), 95% CI: 3·8–12·3) and 6·6% (11/166), 95% CI: 3·3–11·5, Table 1) were diagnosed with syphilis and HBV co-infections, respectively. The risk of being diagnosed with a recent HIV infection significantly increased (IRR: 8·56, 95% CI: 2·94–24·93, P < 0·001, model 2) with a positive HBV test relative to those testing negative. To the contrary, in model 2, results suggest a near equal likelihood of HIV incidence among young people presenting with a seropositive syphilis test (IRR:1·23, 95% CI: 0·18–8·28, P = 0·84) compared with individuals testing negative, even though the statistic was not significant. We further observed that the two co-infections were uniquely detected in samples with HIV RNA above 10,000 copies/ml.

**Knowledge of HIV-positive Status among Newly HIV Infected Adolescents**

Further analysis shows that one in every five (19·3% [32/166], 95% CI: 13·57–26·11) adolescents testing with recent HIV infections in the survey were already aware of their HIV seropositive status. In spite of this verity, laboratory diagnostics did not detect ARVs in their blood samples an indication they had not initiated antiretroviral treatment. Statistically significant associations (= 5·659; P < 0·02) between knowledge of one's HIV-positive status and age were also established. Older adolescents aged 20–24 years had greater awareness of their HIV-positive status (12·7% [21/166]; 95% CI 8·0–18·7) than younger adolescents aged 15–19 years (1·8% [3/166]; 95% CI 0·37–5·2) on awareness of an individual’s HIV-positive status.

**Model Estimation Results**

Even if 85·5% of participants did not know they had HIV, older adolescents were relatively less aware(38·7% [89/166]; 95% CI: 45·7–61·4) than younger adolescents (31·9% [53/166]; 95% CI: 24·9–39·6, Figs. 4a and 4b.). Within age and sex categories, more younger males (88·9% [24/27], 95% CI 70 84–97 65, Fig. 4a.) and younger females (84·9% [118/139], 95% CI: 77·83–90·40) were unaware of their HIV-positive serostatus than their older ones. Besides sex, Figure:4b shows that adolescents in urban areas were less inclined to knowing they had the virus at 50% (83/166; 95% CI: 42·2–57·8) than those residing in rural areas (35·5% 95%, 95% CI: 28·3–43·3).
| Predictors               | Model 1† | Model 2‡‡ | Model 3+++ |
|-------------------------|----------|-----------|------------|
|                         | IRR      | P-Value   | 95% CI     | IRR      | P-Value   | 95% CI     | IRR      | P-Value   | 95% CI     |
| Age                     | 1·00069  | 0·000     | 1·000–1·001| 1·06343  | 0·369     | 0·930–1·216| 0·984041 | 0·837     | 0·844–1·147|
| Sex                     |          |           |            |          |           |            |          |           |            |
| Female                  | 2·27921  | 0·000     | 2·277–2·281| 0·39227  | 0·011     | 0·190–0·809| 0·486252 | 0·044     | 0·241–0·980|
| Male                    | Ref. b   | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Active syphilis c       |          |           |            |          |           |            |          |           |            |
| Positive                | 0·292607 | 0·162     | 0·0522–1·640| 1·21842  | 0·840     | 0·179–8·283| 0·795605 | 0·735     | 0·212–2·990|
| Negative                | Ref.     | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Hepatitis B status      |          |           |            |          |           |            |          |           |            |
| Positive                | 7·66655  | 0·018     | 6·594–41·371| 8·56390  | 0·000     | 2·942–24·931| 6·86583  | 0·007     | 1·702–27·695|
| Negative                | Ref.     | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Marital status          |          |           |            |          |           |            |          |           |            |
| Married                 | 4·07027  | 0·000     | 4·067–4·073| 3·15757  | 0·037     | 1·071–9·313| 2·76422  | 0·117     | 0·774–9·869|
| Not married             | Ref.     | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Residence               |          |           |            |          |           |            |          |           |            |
| Rural                   | 2·37048  | 0·000     | 1·524–3·687| 3·89909  | 0·001     | 1·762–8·631| 3·10161  | 0·003     | 1·488–6·465|
| Urban                   | Ref.     | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Wealth quintile         |          |           |            |          |           |            |          |           |            |
| Lowest                  | Ref.     | ..        | ..         | Ref.     | ..        | ..         | Ref.     | ..        | ..         |
| Second                  | 0·59644  | 0·182     | 0·279–1·274| 1·01981  | 0·982     | 0·194–5·366| -        |           |            |

†Model 1 is a bivariate model unadjusted for other variables. It examines association between each predictor variable and the outcome variable.

‡‡Model 2 is a full controlled model with robust standard errors (SEs) in which inclusion into the model for all variables and covariates was contingent on passing the 0.2 p-value inclusion criterion in model 1 are included. Hence, the variable, aware of one's HIV status was excluded.

+++Model 3 with robust SEs estimation include only statistically significant covariates. The restriction is relaxed for the four main predictors. Three variables, wealth quintile, ever tested for HIV and aware of one's HIV-positive status are not included.
### Model 1†

| Variable                  | Coefficient | Standard Error | Lower CI | Upper CI | Model 2‡‡ | Model 3‡‡‡ |
|---------------------------|-------------|----------------|----------|----------|-----------|-----------|
| Middle                    | 0.24992     | 0.002          | 0.106–   | 0.589    | 0.70233   | 0.153–    |
| Fourth                    | 0.33639     | 0.003          | 0.164–   | 0.690    | 1.12396   | 0.264–    |
| Highest                   | 0.31899     | 0.002          | 0.156–   | 0.654    | 2.42966   | 0.562–    |
| Education                 |             |                |          |          |           |           |
| No education              | Ref.        | ..             | ..       | Ref.     | ..        | Ref.      |
| Primary                   | 5.14169     | 0.027          | 1.202–   | 21.985   | 10.338    | 2.681–    |
| Secondary                 | 1.74790     | 0.442          | 0.421–   | 7.260    | 7.35867   | 1.922–    |
| Higher than secondary     | 1.40491     | 0.671          | 0.292–   | 6.761    | 8.99769   | 1.557–    |
| Number of sex partners    |             |                |          |          |           |           |
| One sex partner           | Ref.        | ..             | ..       | Ref.     | ..        | Ref.      |
| No sex partner            | 0.28871     | 0.011          | -1.296–  | 0.164    | 0.79571   | 0.409–    |
| More than one sex part.   | -2.0569     | 0.000          | -3.081–  | 1.0326   | 0.19566   | 0.068–    |
| Ever tested for HIV       |             |                |          |          |           |           |
| Yes                       | 1.56962     | 0.055          | 0.991–   | 2.486    | 0.95721   | 0.429–    |
| No                        | Ref.        | ..             | ..       | Ref.     | ..        | -         |
| Aware of one’s HIV status |             |                |          |          |           |           |
| Yes                       | 1.25719     | 0.840          | 0.137–   | 11.542   | -         | -         |
| No                        | Ref.        | ..             | ..       | -        | -         | -         |

†Model 1 is a bivariate model unadjusted for other variables. It examines association between each predictor variable and the outcome variable.

‡‡Model 2 is a full controlled model with robust standard errors (SEs) in which inclusion into the model for all variables and covariates was contingent on passing the 0.2 p-value inclusion criterion in model 1 are included. Hence, the variable, aware of one’s HIV status was excluded.

‡‡‡Model 3 with robust SEs estimation include only statistically significant covariates. The restriction is relaxed for the four main predictors. Three variables, wealth quintile, ever tested for HIV and aware of one’s HIV-positive status are not included.

1. a. IRR – These are the incidence rate ratios for the negative binomial regression, showing the rate at which new HIV infections occurred.
2. b. Ref – refers to the variable category used as reference in the specified regression model.
3. c. Active syphilis variable meant the result for the simultaneous presence of antibodies against non-Treponemal and Treponema pallidum antigens clinical test.

We found evidence that incidence of new HIV infections is significantly different between males and females. Increased likelihood of new HIV infection was significantly associated with adolescent girls than boys (IRR 2.30; 95% CI: 2.27–2.28), p < 0.001; Table 2, model 1).

Younger participants had lower relative risk of new infection than older participants (IRR 1.00; 95% CI: 1.00–1.001), p < 0.001; Table 2, model 1). However, associations between age and new HIV infections were completely attenuated when adjustment for covariates was performed (Table 2, model 2 and model 3). Further independent associations were seen in individuals with active syphilis infection (IRR 0.29; 95% CI: 0.05–1.64; model 1), individuals in lower brackets of the wealth quintile (IRR 0.59; 95% CI: 0.28–1.27; model 1) and those with more than one sexual partner in past 12 months (IRR 0.26; 95% CI: 0.09–0.77; model 1). Although mainly consistent in showing decreased likelihood of incidence of HIV infection, but most of these associations did not attain statistical significance.
High educational attainment was associated with high relative risk of new HIV infection by up to 12.8 times (95% CI: 2.09–79.00, p < 0.01; model 3) among those with higher than secondary education compared to individuals with no education. Other socio-behavioural covariates strongly associated with elevated relative risk were; adolescents aware of their HIV-positive status (IRR 1.26; 95% CI: 0.014–11.54; model 1), individuals that had previously tested for HIV (IRR 1.57; 95% CI: 0.99–2.49; model 1), those reporting one sex partner in past 12 months (IRR 2.07; 95% CI: 1.18–3.65; in all three models), those married or cohabiting (IRR 3.16; 95% CI: 1.07–9.31; model 2) and adolescents residing in rural areas (IRR 3.90; 95% CI: 1.76–8.63; model 2 and model 3). With an exception of the variables “aware of one’s HIV status” and “ever tested for HIV”, all estimates indicate statistically significant associations.

**Discussion**

Population-specific studies on new HIV infections are of great public health importance because they offer policy makers and planners definitive insights into the prevalence of the disease burden otherwise shrouded in population aggregation. Studies like ours aid in fully describing the epidemic, monitoring transmission patterns and prioritizing HIV prevention efforts targeted to specific groups such as the adolescent population. Recently infected people with acute HIV infections contribute disproportionately to HIV transmission. Identifying individuals with new infections could have important implications at individual and public health levels. Our analysis quantified the incidence of new HIV infections among adolescents aged 15–24 years relative to standardized virologic and immunological outcomes. We also examined clinical and a range of socio-demographic parameters to uncover characteristics evidently correlated with new infections. Our results highlight that new HIV infections in adolescents are associated with co-infections including testing positive for hepatitis B virus (IRR 8.6 [2.9–24.9]), being female (IRR 1.9 [1.60–2.24]) than male (IRR 0.37 [0.24–0.54]), and being younger, between 15–19 years (IRR 1.4 [0.46–4.21]), than older (20–24 years) at IRR 0.71 (0.24–2.15). Controlling for socio-demographic factors shows that being married or cohabiting, having higher than secondary education, belonging to a higher than middle wealth quintile, and being a rural resident were associated with increased risk for HIV incidence. Among behavioral attributes, having previously tested for HIV and reporting one sex partner in the past 12 months were associated with increased likelihood for infection.

Our study was not short of limitations. Accuracy in estimating HIV incidence and acute HIV infection in cross-sectional studies is sharply queried for suboptimal performance. The EIA and BED assays used in this study in the determination of recent HIV infections are used only on HIV seropositive specimens. This method employed the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS). However, this methodology and serologic assays therein have previously shown to contain substantial limitations, including biological, epidemiological, and statistical confounders (33). Additionally, although the EIA and BED assays used in our study are widely known, acceptance and use of these assays has been intensely disputed for its tendency to overestimate incidence (20). Therefore, we, may have overestimated the incidence of new HIV infections in adolescents and resulting associations with other parameters. Although we controlled for socioeconomic factors that have previously been linked to HIV prevalence; it is possible that residual confounding remains for parameters not considered in model estimation. Future studies should consider expanding confounders to augment appropriateness in determining factors associated with new HIV infections in sub-population groups. Nevertheless, granting that our study focuses on the population 15 to 24 years, the method used for new HIV infection estimation draws its strength from global recommendations stating that tendencies in prevalence among adolescents 15 to 24 years be utilized as proxy measures for calculating HIV incidence (21, 34). This is because sexual debut in this age group is expected to be recent such that prevalence closely reflects recent infections.

During the first few weeks following HIV-1 seroconversion, HIV RNA viral load surges, which poses considerable risk of HIV transmission (35, 36). Our study results show that the mean HIV RNA was 164,183 copies/ml (64,886 – 263,480 copies/ml, Fig. 2b.), three times more than the 50,000 copies/ml HIV RNA limit in someone not taking treatment. Which suggests that newly infected adolescents, on average, had high HIV RNA levels at point of first diagnosis. There is strong evidence from research that disease progression is substantially escalated in patients with HIV-1 RNA levels > 100,000 copies/ml, regardless of CD4 + T-cell count (37). Strongly associated with baseline viral load levels is treatment efficacy and response to therapy. HIV RNA levels greater than 150,000 copies/ml correspond to 1.5 times increased likelihood of treatment failure which is the ability to decrease the viral load to less than 50 copies/ml (37).

Variations in viral load between males and females were also clearly noticeable. In congruence with several studies (38–41), we found higher proportions of HIV RNA viral load ≥ 50,000 copies/ml in males (59.3% [16/27], 95% CI: 38.8–77.61) than females (45.3% [63/139], 95% CI: 36.9–54.0). Consistent with results from our descriptive analysis which indicate lower proportions of females with HIV RNA viral load ≥ 50,000 copies/ml than males, model estimation results also confirm significant independent associations for both age and sex. Increasing age was positively associated with new infections (P < 0.001, model 1), although the correlation with age was abated in controlled models (model 2 & 3). Irrespective of viral load levels, female adolescents were 2.3 times (P < 0.001, model 1) at increased risk of HIV seroconversion in relation to males. These differentials are substantively documented in previous studies highlighting up to 50% lower HIV RNA viral load and higher CD4 + T-cell counts in HIV-1 infected women than men soon after seroconversion (39, 40, 42–44). However, other studies confirm attenuation of the sex effect in advanced stages of infection (41, 45). Moreover, increasing age at seroconversion has been associated with increased risk of speedy immunologic deterioration and high virologic replication (35, 37). Touloumi and colleagues verified that older age at the time of HIV antibody
seroconversion was associated with shortened period to AIDS indicated by steepest (most negative) HIV RNA level and CD4 + T-cell slopes in the younger population groups like adolescents (46).

Extensive loss of mucosal CD4 + T cells occurs in the early stages of acute HIV infection, once this biomarker of immunologic potential falls below 500 cells/mm3, much of the immune reserve is wrecked and infected persons become susceptible to opportunistic infections. In this study, we found that 62% (95% CI: 54·65–69·46) of recently HIV infected adolescents had CD4 + T-cell count below 500 cells/mm3 with half of these young people aged 15–24 years diagnosed with CD4 + T-cell count of 479 cells/mm3 (95% CI: 445–513·6) from the normal functional range of between 500 cells/mm3 and 1500 cells/mm3. As the primary target of HIV, depletion of CD4 + T-cells acutely constrains the host response capacity. HIV infects activated cells, causing the T-cells directed against the virus to be at greatest risk of infection (35, 47). From the point of acquisition to AIDS, disease progresses has formerly been linked to baseline CD4 + T-cell count, HIV RNA levels and a number of other determinants including age and sex (48, 49). In corroborations, our analysis finds that 63% of males aged 15–24 with CD4 + T-cell counts ≤ 500 cells/mm3 coincided with 59.3% of them having HIV RNA ≥ 50,000 copies/ml compared with 61% and 45.3% respectively, of their female equivalents. Additionally, 64.5% and 57.1% of adolescents aged 20–24 and 15–19 years separately, had CD4 + T-cell count below 500 cells/mm3. These results imply that a large proportion of adolescents testing positive for HIV, especially males, already have their immune system potency far deteriorated. In line with our findings, Bosch and colleagues found that younger age was a significant independent predictor of greater CD4 + T-cell count (42). Other studies have verified that age at seroconversion and HIV RNA level are associated with the CD4 + T-cell count at baseline and its subsequent slope to the ultimate disease syndrome (36, 46, 47). Further estimates highlight that within two years of contracting HIV, older individuals and those with the highest HIV RNA levels during early infection experience the most severe depletion of CD4 + T-cells (43).

Co-infection, particularly with HBV, was one factor associated with incidence of HIV in young people. We identified that 2% (95% CI: 0·41–5·70) of new HIV infections in the population 15–24 years also tested positive for HBV. In our confounder-adjusted models, HBV – positive adolescents were 8·6 times (P < 0·001, model 2) at increased risk of new infection relative to HBV – negative adolescents in the same age stratum. Because HBV is more infectious and adolescence is a period of increased sexuality, most infections have been found to occur in adolescents and young adults (50). Our findings align with several studies in Africa that have established increased Hepatitis B virus vulnerability in HIV-positive persons (51–55). Clinically, HBV belongs to a variety of heterologous viruses that have been shown to enhance HIV replication (36). Therefore, individuals who are HIV-positive and also test positive for HBeAg are more likely to transmit both viruses (25, 50). The HIV– HBV co-infection augments risk of morbidity, antiretroviral therapy-related hepatotoxicity and mortality beyond those caused by either infection alone (56, 57). Similar to HBV results, syphilis co-infection was also estimated at 2%. Adolescents who tested positive for syphilis antibodies were 1·2 times (P < 0·84, model 2) more likely to contract HIV even though correlation was not statistically significant. Both HBV and syphilis co-infections were observed to occur at HIV RNA > 10,000 copies/ml RNA suggesting increased susceptibility during acute infection. Syphilis has been associated with high-risk sexual behaviour, increasingly prevalent in adolescence (58) and reported to be one of the more frequently occurring infection among in HIV infected people (54).

Conclusion

Our study suggests new HIV infections in adolescents are first diagnosed at high HIV RNA and low CD4 + T-cell count increasing the risk of transmission and likelihood of treatment failure with reduced ability to achieve viral suppression. Sex differentials highlight disproportionate susceptibility of female adolescents to infection than males. Additionally, confounder-adjusted models ascertained that adolescents who were married or cohabiting, were positive for hepatitis B virus, were rural residents and had attained higher than secondary education emerged as the strongest correlates of new infections. While the epidemic continues to spread within the adolescent population, HIV transmission and acquisition will become more complex and greater proportions of adolescents will be infected by their regular partners, especially rural ones. Which implies an increasing need for interventions targeted at stable partnerships, intensified public health campaigns specific for rural adolescents and population segmented preventive health services. However, adolescents being the less prioritized population group in health response and routinely overlooked in national plans, the advent of the COVID-19 pandemic poses a major threat to reversing minimal preventive gains so far achieved in adolescent HIV prevention.

Abbreviations

ART Antiretroviral Therapy
CD4 T-Cell CD4 T Lymphocyte
HIV Human immunodeficiency Virus
HIV RNA HIV Ribonucleic acid
HBTC HIV Home-Based Testing and Counselling

HBV Hepatitis B Virus

STARHS Serologic Testing Algorithm for Recent HIV Seroconversion

ZAMPHIA Zambia Population-based HIV Impact Assessment

Declarations

Authors’ Contribution

TNM and NM developed the manuscript concept. TNM and NM sourced data, re-coded data, conducted statistical analysis and interpreted analysis results. TNM drafted initial manuscript. XZ, SK, CG, XQ, KS and JW reviewed methods, analysis results and interpretation. All authors edited and approved the final content of the manuscript before submission.

Data sharing

Data used in this article are available to bona fide researchers on request from Zambia’s Ministry of Health (www.moh.gov.zm) through email (info@moh.gov.zm).

Declaration of interests

All authors declare no competing interests.

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Funding

No funding was received to produce the manuscript.

Authors’ contributions

TNM and NM developed the manuscript concept. TNM and NM sourced data, re-coded data, conducted statistical analysis and interpreted analysis results. TNM drafted initial manuscript. XZ, SK, CG, XQ and KS reviewed methods, analysis results and interpretation. All authors edited and approved the final content of the manuscript before submission.

Ethics declaration

Since this study was based on secondary individual de-identified dataset, no ethical approval was required. However, ethical approval for the protocol for ZAMPHIA Population-based survey on which the study is based was obtained from the Tropical Diseases Research Centre (TDRC) Zambia (FWA00003729), the Centers for Disease Control and Prevention (CDC), Columbia University IRB (FWA # 00002636), and WESTAT (FWA # 00005551).

Consent for publication

Not applicable

Acknowledgements

Not applicable

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Figures

**Figure 1**

STARHS biomarker detection schema used in estimating new HIV infections
Figure 2

Viral Load and CD4 T-Cell Count for New HIV Infection in Adolescents

Figure 3

CD4 T-Cell Count of Newly Infected Adolescents
Figure 4

Figure 4