RESEARCH ARTICLE

Incidence of HIV and the Prevalence of HIV, Hepatitis B and Syphilis among Youths in Maputo, Mozambique: A Cohort Study

Edna Omar Viegas1,2,3*, Nelson Tembe1,2,3*, Eulália Macovela3,4, Emília Gonçalves3,4, Orvalho Augusto5, Nália Ismael1, Nádia Sito1, Caroline De Schacht1, Nilesh Bhatt1, Bindiya Meggi1, Carolina Araujo3, Eric Sandström8, Gunnel Biberfeld5,6, Charlotta Nilsson2,5,6, Sören Andersson9, Ilesh Jani1, Nafissa Osman3,4

1 Instituto Nacional de Saúde, Maputo, Mozambique, 2 Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden, 3 Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique, 4 Hospital Central de Maputo, Maputo, Mozambique, 5 Public Health Agency of Sweden, Solna, Sweden, 6 Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, 7 Elizabeth Glaser Pediatric AIDS Foundation, Maputo, Mozambique, 8 Department of Education and Clinical Research, Karolinska Institutet, Södersjukhuset, Stockholm, Sweden, 9 Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

* These authors contributed equally to this work.
* ednaviegas@gmail.com

Abstract

Background

Prevalence of HIV in Mozambique among individuals aged 15–49 years is 11.5%. The HIV prevalence is higher in women than in men across the country, peaking at ages 25–29 years and 35–39 years, respectively. In this study, we aimed at determining the prevalence and incidence of HIV, prevalence of Hepatitis B (HBV), and prevalence of syphilis in youths. We also characterized a cohort of youths for future participation in phase I/II HIV vaccine trials.

Methods

The study was conducted at a youth clinic in Maputo Central Hospital from August 2009 to October 2011. Youths of both genders aged 18–24 years (n = 1380) were screened for HIV using a sequential algorithm of two immunochromatographic assays, HBV using an enzyme linked immunosorbant test, and syphilis using a treponemal immunochromatographic strip test. The HIV seronegative participants (n = 1309) were followed-up for 12 months with quarterly study visits. The clinical and behavioral data were collected using structured questionnaires. The HIV seroconversions were confirmed by a molecular assay.

Results

The study population was female dominant (76.8%). All participants had a formal education, with 44.6% studying for technical or higher education degrees. The mean age at sexual debut was 16.6 years (SD: ±1.74), with 85.6% reporting more than one sexual partner in
life. The screening showed the prevalence of HIV, HBV, and syphilis at 5.1% (95% CI: 3.97–6.31), 12.2% (95% CI 10.5%–14.0%), and 0.36% (95% CI 0.15%–0.84%), respectively. The HIV incidence rate was found to be 1.14/100 person years (95% CI: 0.67–1.92). Retention rates were stable throughout the study being 85.1% at the last visit.

**Conclusion**

Incidence of HIV in this cohort of youths in Maputo was relatively low. Also, the prevalence of HIV and syphilis was lower than the national values in this age group. However, the HBV prevalence was higher than in previous reports in the country.

**Introduction**

HIV/AIDS continues to cause high morbidity and mortality, particularly in sub-Saharan Africa [1]. In 2012, 35.3 million people were infected by HIV worldwide. Mozambique has the fifth highest prevalence of HIV in the world, with 11.5% of the 15–49 years old population infected with HIV [2,3]. Women have a higher prevalence than men (13.1% vs 9.2%) [3]. In Mozambique, the peak of HIV prevalence is found in women aged 25–29 years (16.8%) and in men aged 35–39 years (14.2%) [3]. In 2012, the world HIV prevalence in youths aged 15–24 was 0.8% and 4.7% in Sub-Saharan Africa [4]. In Mozambique, the prevalence in this age group, in 2009, was 4.2% (4.8% and 3.5% in women and men, respectively) [3]. In sub-Saharan Africa, women face significantly higher risk of HIV acquisition and are infected at earlier ages. Factors contributing to a higher rate of HIV infection in women include gender inequalities leading to unequal power relationships, unequal access to education and economic opportunities, [1] and biological factors.

Sexually transmitted infections (STIs), including hepatitis B and syphilis, are important public health concerns and constitute risk factors for HIV acquisition [5]. Worldwide, it is estimated that more than two billion people have been infected with the hepatitis B virus (HBV) [6], a disease that can lead to chronic infection, development of liver cirrhosis and hepatocellular carcinoma. Sub-Saharan Africa is considered by the World Health Organization (WHO) as a highly endemic area for HBV [7]. Transmission of HBV in sub-Saharan Africa commonly occurs during childhood. Other modes of transmission such as sexual and parenteral are also important, particularly in areas where unprotected sex is a common practice. In Mozambique, studies among blood donors demonstrate that HBV prevalence in the northern and southern regions of the country is 10.6% [8] and 9.3% [9], respectively. Syphilis is an important cause of morbidity and mortality, especially in pregnant women and infants [10]. In Mozambique, the national prevalence of syphilis in pregnant women in 2011 was 2.2%, ranging from 1.2% to 8.2% [11] in the southern and northern region, respectively.

Youth clinics (“SAAJ, Serviço Amigo do Adolescente e Jovem”) have been established throughout Mozambique with the aim of providing sexual and reproductive health services, and to encourage behavior change through peer education. A study conducted by Melo et al. [12] in 2002–2003 demonstrated that youths attending a youth clinic in Maputo had high level of awareness of STIs including HIV, and that the HIV prevalence in this group was lower than the estimated prevalence in the general population [3]. Behavioral change is one of the targets of the National Aids Council of Mozambique [13]. However, from 2003 to 2009, disturbing signs of increasing sexual risk behavior among young Mozambicans aged 15–24 years have emerged [1]. Although development of a safe, effective, and affordable preventive vaccine is far in the horizons, it may be the best long-term hope to control the HIV/AIDS pandemic in resource-limited settings [14].
In order to understand the dynamics of HIV transmission, data on new infections is needed [15,16]. This is particularly important in the age group where most of the transmissions are occurring. Mozambique has an expansive age pyramid, i.e., the majority of the population in the country is young. Approximately one third of the population is within the age group of 15–49 years [17], where the highest rate of HIV infections is occurring. Although HIV prevalence is well documented in Mozambique, information on HIV incidence in young population is lacking. We have studied a cohort of young people at a clinic providing services to adolescents and youths ("SAAJ"-clinic) in Maputo. The aims of the present study were to: 1) describe the socio-behavioral characteristics of this population, 2) determine the prevalence and incidence of HIV infection, 3) determine the prevalence of hepatitis B and syphilis, and 4) assess the suitability of the cohort for possible participation in phase I/II HIV vaccine trials.

Subjects and Methods

Ethics statement

This study was approved by the National Health Bioethics Committee of Mozambique (reference 148/CNBS on May 8, 2009) and followed the GCP ICH guidelines. Written informed consent was obtained from each participant prior to conducting any study procedures.

Study population

This prospective cohort study was conducted at the Maputo Central Hospital, Mozambique, between August 2009 and October 2011. The participants were recruited and followed-up at an outpatient youth clinic specializing in provision of sexual and reproductive health services including STI/HIV prevention and care. Youths aged 18–24 of both genders and residing in Maputo were invited to participate in the study. Information sheets were handed to those who showed interest in taking part in the study. Trained research staff conducted individual detailed review of study procedures. All questions regarding study participation were addressed prior to signing the informed consent.

Screening and enrolment

At baseline, a face-to-face interview to assess knowledge, attitude and practices (KAP survey) in relation to HIV and STIs was conducted by trained peer counselors using structured questionnaires. The socio-demographic data and clinical history were obtained by the study nurses who also performed physical examination on all the study participants. Screening for HIV, HBV and syphilis was done before enrolment and dried blood spots (DBS) were collected and stored. Pre- and post-HIV test counseling was offered individually and confidentially according to the national guidelines. Male and female condoms were provided to all the participants. Participants with negative or indeterminate HIV test results were enrolled in the longitudinal HIV incidence study, which began on the same day. Locator information such as telephone numbers and residential address were also collected by the study team.

Follow-up visits

Upon enrolment, participants were asked to return to the study site on a quarterly basis over the course of 12 months, i.e., for three follow-up visits. The visit window was determined to be +/-8 weeks. At each follow-up visit the participants underwent: 1) a one-to-one interview to assess the HIV-related risk behaviors using a structured questionnaire, 2) clinical examination to identify possible signs and symptoms of acute HIV infection, 3) HIV counseling and testing, and 4) STIs risk reduction counseling. Male and female condoms were provided at all study visits.
At each visit, DBS were collected and stored at the study laboratory for the confirmation of time to event, i.e., time between a negative and positive HIV result. Contact details and locator information were updated at each follow-up visit. Participants failing to attend a scheduled visit were contacted by phone the following day. Active tracing was done whenever phone contact was not successful. A loss to follow-up was defined as a participant who did not attend the remaining follow-up study visits and was not reachable by phone or active tracing. Participants who expressed a desire to discontinue their participation in the study were included under the category of study discontinuation. Seroconverting subjects were defined as those who presented with a non-reactive HIV rapid test on a study visit followed by a reactive result on the subsequent visit.

**Laboratory testing**

HIV testing followed the national algorithm [18]. Youths were tested using an on-site sequential algorithm of two immunochromatographic assays: the Determine HIV-1/2 (Abbott Laboratories, Illinois, USA), followed by a confirmatory test UniGold HIV-1/2 (Trinity Biotech, Bray, Wicklow, Ireland). An individual was considered HIV infected when both assays were reactive. An indeterminate HIV test result was defined as a reactive Determine test followed by a non-reactive UniGold assay. As a means of determining the timing of HIV infection in a subject with a reactive HIV rapid assay on a follow-up visit, the DBS samples collected on Whatman filter papers from the previous visits were tested using a molecular assay (Roche Amplicor HIV-1 DNA test, version 1.5, Roche Molecular Diagnostics, Branchburg, NJ) [19].

HIV-1 viral load was measured using a COBAS Taqman48 analyzer (Roche Molecular Diagnostics, Mannheim, Germany), and CD4+ T-cells count was determined using a Becton Dickinson FACSCalibur instrument (Biosciences Corp, NJ, USA). Both tests were performed in all HIV infected participants on the day of diagnosis.

Serum samples were collected for the hepatitis B surface antigen (HBsAg) screening. Tests were performed using an enzyme linked immunosorbent assay (HUMAN GmbH, Wiesbaden, Germany). Syphilis testing was performed on-site, in whole blood, using a treponemal immunochromatographic strip test (SD BIOLINE Syphilis 3.0, Standard Diagnostics, Kyonggi-do, Korea).

**HIV/Syphilis treatment and Hepatitis B referral**

Participants diagnosed with an HIV infection during the study received HIV care and treatment services at the youth clinic. The CD4+ T-cells count and the HIV-1 viral load were determined in each incident case and made available for clinical follow-up. Participants with a reactive syphilis test were treated at the study site per national treatment guidelines and those diagnosed with HBV infection were referred for clinical management at the Gastroenterology Department of Maputo Central Hospital.

**Data processing and statistical analysis**

Data was entered in a MySQL database version 5.1 with a frontend designed in Microsoft Office Access 2007. Validating rules and skipping patterns were implemented to ensure data quality. Data was exported to Stata 12 (StataCorp 2011, Stata Statistical Software: Release 12, College Station, TX: StataCorp LP) for statistical analyses. Descriptive statistics were used to summarize the baseline demographic and behavioral characteristics. Categorical variables were expressed in percentages, and continuous data as means with respective standard deviations (SD). The significance level was set at 5%. For baseline data, the unadjusted odds ratios and their 95% confidence intervals for each cofactor were calculated. Cofactors with a p-value less than 0.2 on either a Pearson or Fisher's exact chi-square were included on the logistic multivariate analysis using a stepwise backward regression. At each step, variables were tested to be
removed from the model at a significance level of 0.10 using the likelihood-ratio test. Two models were built, one for overall participants, and one per gender.

The HIV incidence rate (IR) was calculated by dividing the number of new HIV cases by the person-years (PY) of the cohort. Uninfected participants that attended only one study visit (visit 1) were considered censored at the 60th day of follow-up. The IR was expressed as number of cases per 100 PY of follow-up, and its 95% exact Poisson confidence intervals (CI) were reported. Hazard rates and their robust confidence intervals were reported for each cofactor. The retention rates per visit were calculated by dividing the number of participants who attended a study visit by the expected number, and was expressed in percentages. HIV seroconversions were excluded from the denominator for the following visit.

Results
Baseline demographic and behavioral characteristics
A total of 1380 youths were enrolled in the HIV prevalence study, out of which 320 (23.2%) were males and 1060 (76.8%) were females. The demographic characteristics of all screened participants are presented in Table 1. The mean age of the participants was 20.9 years (SD: ±1.71) with males being older than females (21.4 vs 20.7, p <0.001). Almost all the participants (98.6%) were single, and approximately half (55.4%) of them had primary or secondary education. There were no illiterate participants.

Sexual behavior characteristics at screening are listed in Table 1. The mean age at sexual debut was 16.6 years (SD: ±1.74). Male participants reported to have initiated sexual activity earlier than female participants, 16.0 years (SD: ±2.16) vs 16.8 (SD±1.55), respectively, p<0.001, with 68.8% of youths reporting to have initiated sexual activity before the age of 18 years. A total of 1182 (85.7%) participants reported to have had more than one sexual partner in life, and 270 (19.6%) reported multiple sex partners six months prior to the study participation. The number of participants that reported more than one sexual partner in the previous six months was significantly higher in male than in female participants (38.8% vs 13.8%, p<0.001). About one third of youths (29.5%) reported at least one episode of STI in life and 86.0% of them were females (p<0.001). Use of condom during the last reported sexual intercourse was significantly different between genders (p = 0.008), being 63.2% for females and 71.3% for males. Approximately, half of the study participants (49.9%) reported use of alcohol (69.1% vs 44.1% in men and women, respectively, p<0.001), while use of tobacco, injectable drugs and/or other drugs was reported only by a few (3.44% vs 0.94% in men and women, respectively) (data not shown).

HIV prevalence
The overall HIV prevalence at the time of screening was 5.1% (71 infections; 95%CI: 3.97–6.31; Table 2), and the prevalence was significantly higher in women than in men (5.8% vs 3.1%, p = 0.018). Data on HIV prevalence by gender in relation to socio-demographic and behavioral aspects are presented in supplementary S1 Table and S2 Table. For each year of age, the odds to be HIV-infected increased by 81% in men (p = 0.020) and by 37% in women (p<0.001). Although the HIV infections were more frequent (73%) in youths with lower educational level, analysis by gender demonstrated that the impact of education was only significant in the female population (p = 0.006). Sexual debut at age below 18 was associated with higher HIV prevalence in females (p = 0.005). No association was found between HIV infection and the number of sexual partners in life or with the number of sexual partners six months prior to the study participation. Men who reported to have had sexually transmitted infections had a significantly higher HIV prevalence (p = 0.033). This was not observed in the female population.
Participant retention and HIV incidence

Participants enrolled in the HIV incidence study (n = 1309) attended a total of 3414 follow-up visits, which accounted for a total of 1229.78 person years of follow-up. The average years of follow-up were 0.94 (range 0.16–1.17). Fourteen seroconversions occurred throughout the study, four within the first four months of the study initiation, six between the fifth and eighth
The HIV incidence rate was 1.14/100PY (95% CI: 0.67–1.92). All incident infections occurred in the female participants, and the HIV incidence in women was 1.49/100 Women Years (WY) (95% CI: 0.67–2.61).

### Table 2. Baseline socio-demographic and behavioral characteristics and HIV prevalence.

| Characteristic                     | Total                  | HIV negative | HIV positive | Unadjusted | Adjusted |
|------------------------------------|------------------------|--------------|--------------|------------|----------|
|                                    | N          | %       | N          | %       | Prevalence | OR CI 95% | p        | OR CI 95% | p        |
| Total Screened                     | 1380       | 1309    | 71         | 5.1%    |            |          |          |          |          |
| Gender                             |            |         |            |         |            |          |          |          |          |
| Male                               | 320        | 23.2%   | 310        | 23.7%   | 10        | 14.1%    | 3.1%    | 0.53      | 0.27–1.04 | 0.066    |
|                                    |            |         |            |         |            |          |          | 0.43      | 0.21–0.87 | 0.018    |
| Female                             | 1060       | 76.8%   | 1000       | 79.3%   | 61        | 85.9%    | 5.8%    |            |          |          |
| Age (change in a year)             | -          | -       | -          | -       | -         | -        | -       | 1.29      | 1.12–1.48 | < 0.001* |
| Marital Status                     |            |         |            |         | -         | -        | -       | 1.43      | 1.23–1.65 | < 0.001  |
| Education                          |            |         |            |         | -         | -        | -       | -         | -         |          |
| Primary and Secondary              | 764        | 55.4%   | 727        | 54.1%   | 52        | 73.2%    | 6.8%    |            |          |          |
| Technical training                 | 278        | 20.1%   | 271        | 20.4%   | 11        | 15.5%    | 4.0%    | 0.33      | 0.16–0.71 |          |
| University degree                  | 338        | 24.5%   | 329        | 25.4%   | 9         | 11.3%    | 2.4%    | 0.56      | 0.29–1.10 |          |
| Occupation                         |            |         |            |         | -         | -        | -       | 0.29      | 0.14–0.62 | 0.002    |
| Student                            | 1329       | 96.3%   | 1261       | 96.3%   | 68        | 95.8%    | 5.1%    | -         |          |          |
| Employed                           | 51         | 3.7%    | 48         | 3.7%    | 3         | 4.2%     | 5.9%    | 1.16      | 0.35–3.82 |          |
| Religion                           |            |         |            |         | -         | -        | -       | -         | -         |          |
| Christian                          | 1246       | 90.3%   | 1185       | 90.5%   | 61        | 85.9%    | 4.9%    | 0.64      | 0.32–1.28 | 0.205    |
| Other                              | 134        | 9.7%    | 124        | 9.5%    | 10        | 14.1%    | 7.5%    |            |          |          |
| Age at sexual debut (years)        |            |         |            |         | -         | -        | -       | 0.743†    |          |          |
| Less than 18                       | 950        | 68.8%   | 894        | 68.3%   | 56        | 78.9%    | 5.9%    | -         |          |          |
| 18 or more                         | 430        | 31.2%   | 415        | 31.7%   | 15        | 21.1%    | 3.5%    | 0.58      | 0.32–1.03 | 0.45     |
| Number of sex partners in life     |            |         |            |         | -         | -        | -       | 0.25–0.82 |          |          |
| 1                                  | 198        | 14.3%   | 190        | 14.5%   | 8         | 11.3%    | 4.0%    | -         |          |          |
| >1                                 | 1182       | 85.7%   | 1119       | 85.5%   | 63        | 88.7%    | 5.3%    | 1.34      | 0.63–2.84 |          |
| Number of sex partners in the last 6 months | 1380 | 99.5%   | 1309       | 99.5%   | 71        | 100.0%   | 5.2%    |            |          |          |
| 0–1                                | 1110       | 80.4%   | 1050       | 80.2%   | 60        | 84.5%    | 5.4%    | -         |          | 0.376    |
| >1                                 | 270        | 19.6%   | 259        | 19.8%   | 11        | 15.5%    | 4.1%    | 0.74      | 0.39–1.43 |          |
| Condom use in the last sexual intercourse | 764 | 55.4%   | 727        | 54.1%   | 52        | 73.2%    | 6.8%    |            |          |          |
| No                                 | 482        | 34.9%   | 456        | 34.8%   | 26        | 36.6%    | 5.4%    | -         |          | 0.759    |
| Yes                                | 898        | 65.1%   | 853        | 65.2%   | 45        | 63.4%    | 5.0%    | 0.93      | 0.56–1.52 |          |
| Alcohol consumption                |            |         |            |         | -         | -        | -       | -         | -         |          |
| No                                 | 692        | 50.1%   | 659        | 50.3%   | 33        | 46.5%    | 4.8%    | -         |          | 0.526    |
| Yes                                | 688        | 49.9%   | 650        | 49.7%   | 38        | 53.5%    | 5.5%    | 1.17      | 0.72–1.88 |          |
| Drug use                           |            |         |            |         | -         | -        | -       | -         | -         |          |
| No                                 | 1373       | 99.5%   | 1302       | 99.5%   | 71        | 100.0%   | 5.2%    |            |          |          |
| Yes                                | 7          | 0.5%    | 7          | 0.5%    | 0        | 0.0%     | 0.0%    |            |          |          |
| Had a STI before                   |            |         |            |         | -         | -        | -       | -         | -         |          |
| No                                 | 973        | 70.5%   | 927        | 70.8%   | 46        | 64.8%    | 4.7%    | -         |          | 0.281    |
| Yes                                | 407        | 29.5%   | 382        | 29.2%   | 25        | 35.2%    | 6.1%    | 1.32      | 0.80–2.18 |          |

* Likelihood Chi-squared test
† Fisher's exact chi-squared test

doi:10.1371/journal.pone.0121452.t002
0.88–2.51). None of the seroconverters had hepatitis B or syphilis at the time of enrolment. There were no associations between gender, age, level of education, religion or sexual behavior and increased risk of HIV acquisition (Table 3). We observed a non-significant trend towards higher risk of HIV acquisition in participants who were married or cohabiting (p = 0.055). The cohort retention was 82.2% in the first quarter, 81.1% in the second quarter and 85.1% in last quarter of the study. Table 4 shows the compliance rates in relation with the socio-demographic characteristics. Approximately two-thirds (73.5%) of the participants completed the study schedule and attended all four study visits. Single and higher educated participants were significantly more compliant than those who were married and less educated (p = 0.049 and p = 0.043, respectively). Students had a better visit compliance rate than those with a formal employment (p = 0.04).

Immunological and virological characteristics of the HIV incident cases
CD4+ T-cells and viral loads were measured in all HIV seroconverters. The median CD4+ T-cell count and viral load were 608 cells/mm³ (IQR 397–648) and 74,182 copies/mL (IQR 22,555–166,825), respectively (data not shown).

Hepatitis B and Syphilis prevalence
Hepatitis B surface antigen was detected in 168 out of 1377 youths (12.2%; 95%CI: 10.5%–14.0%). The number of HBV infections was significantly higher in men (51, 15.9%) than in women (117, 11.1%) (p = 0.02). Among the individuals with HBV infection, eight were HIV positive (4.9%).

Out of 1378 participants, five (0.36%; 95%CI: 0.15%–0.84%) had a positive syphilis test (three female, two male). One individual infected with syphilis was co-infected with HIV, two co-infected with HBV, and one co-infected with both HIV and HBV.
Discussion

In this study we have documented the seroprevalence of three sexually transmitted infections (HIV, HBV and syphilis) among youths in Maputo, Mozambique. The HIV prevalence in the present study (5.1%) was lower than the overall prevalence found in young people aged 19–24
years in 2009 in Mozambique (10.9%) during a community-based survey (INSIDA) [3]. An ante-
natal surveillance round (ANSR) conducted in 2011 demonstrated an overall HIV prevalence
of 13.2% in pregnant women aged 15–24 [20]. In semi-rural areas of southern Mozambique,
the prevalence of HIV was 23.2% in the age group of 18–27 years old men and women in 2010
[21]. Previous results from Melo et al. [12] have also shown a lower HIV prevalence (4%) in
young females aged 15–24 at the same youth clinic. Both our and Melo et al.’s studies have shown that HIV infections decrease with the in-
crease in level of education, suggesting that education has an important role in HIV prevention
[12]. These two studies were conducted at a youth clinic that is centrally located, easily accessi-
able, and where HIV counselling and testing, STI treatment, condom provision and sexual be-
havior education are available free of charge. This could have also contributed to the lower
HIV prevalence documented in both the studies.

Here, we have reported a higher number of HIV infections in women. Similar findings were
reported by INSIDA [3] in Mozambique and in other south-east African countries [22,23,24].
The rate of HIV infections was higher with increasing age, with women being infected earlier
in life compared to men. These findings were also described elsewhere in Mozambique [3] and
southern Africa [25]. The age of sexual debut in women was also identified as a risk for HIV in-
fec tion, corroborating the findings by Hallet et al. [26].

Although adolescents and youths are more prone to risk behaviors such as alcohol abuse
and multiple partners, our study did not show any significant association of alcohol abuse and
multiple partners with increased risk of HIV-infection. Men who reported to have had STIs

| Characteristic                        | Total Completed visits | Total | P      |
|--------------------------------------|------------------------|-------|--------|
|                                       | One        | Two    | Three  | Four    |       |
|                                       | N   | %   | N   | %   | N   | %   | N   | %   |
| Total                                | 70  | 5.3%| 94  | 7.2%| 183 | 14.0%| 962 | 73.5%| 1309 |
| Gender                               |             |       |       |       |       |       |       |       |
| Male                                 | 22 | 7.1%| 24  | 7.7%| 41  | 13.2%| 223 | 71.9%| 310  | 0.423 |
| Female                               | 48 | 4.8%| 70  | 7.0%| 142 | 14.2%| 739 | 74.0%| 999  |
| Age categories (years)               |             |       |       |       |       |       |       |       |
| < 21                                 | 42 | 5.8%| 49  | 6.8%| 98  | 13.6%| 531 | 73.8%| 720  | 0.755 |
| 21 +                                 | 28 | 4.8%| 45  | 7.6%| 85  | 14.4%| 431 | 73.2%| 589  |
| Education                            |             |       |       |       |       |       |       |       |
| Primary and Secondary                | 47 | 6.6%| 59  | 8.3%| 107 | 15.0%| 499 | 70.1%| 712  | 0.043 |
| Technical training                   | 12 | 4.5%| 19  | 7.1%| 36  | 13.5%| 200 | 74.9%| 267  |
| University degree                    | 11 | 3.3%| 16  | 4.8%| 40  | 12.1%| 263 | 79.7%| 330  |
| Marital Status                       |             |       |       |       |       |       |       |       |
| Single                               | 67 | 5.2%| 91  | 7.0%| 181 | 14.0%| 952 | 73.7%| 1291 | 0.049* |
| Married/Cohabitating                 | 3  | 16.7%| 3  | 16.7%| 2  | 11.1%| 10  | 55.6%| 18   |
| Occupation                           |             |       |       |       |       |       |       |       |
| Student                              | 68 | 5.4%| 85  | 6.7%| 178 | 14.1%| 930 | 73.8%| 1261 | 0.040* |
| Employed                             | 2  | 4.2%| 9   | 18.8%| 5   | 10.4%| 32  | 66.7%| 48   |
| Religion                             |             |       |       |       |       |       |       |       |
| Christian                            | 62 | 5.2%| 86  | 7.3%| 163 | 13.8%| 874 | 73.8%| 1185 | 0.805 |
| Other                                | 8  | 6.5%| 8   | 6.5%| 20  | 16.1%| 88  | 71.0%| 124  |

* Fisher’s exact chi-square test p-value

doi:10.1371/journal.pone.0121452.t004
were more at risk for HIV infection, which was not true for women. STIs have been described as a risk factor for HIV infection [27] and the lack of association in women in the present study could be a result of their definition of STIs (normal mucous vaginal discharge could have been interpreted as STI), as well as from lack of probing from the staff conducting the questionnaires.

To the best of our knowledge, this is the first cohort study to assess HIV incidence in youths aged 18–24 in Mozambique. Retention rates in our study were similar to those found in other HIV cohort studies [28] or even higher [29]. These rates were stable throughout the study period showing that efforts from peer counselors and nurses to contact participants and to maintain the participants’ willingness to be involved in the study were successful. The incidence found in this study was 1.14/100PY, which is lower compared to the incidence found in studies in pregnant (4.3/100 WY; 95%CI 0.5–7.2) and post-partum Mozambican women (3.2/100 WY; 95%CI: 2.3–4.5) [30]. Two other cohort studies have shown a high HIV incidence in high risk women aged 18–35 years (4.6/100 WY; 95%CI: 2.7–7.3) [31] and 18–24 years (6.5/100 WY; 95%CI: 4.1–9.9) [32] in southern and central Mozambique, respectively. In South Africa, cohort studies in young women in rural and urban areas reported a high incidence of HIV infection, 6.9/110 WY [28] and 14.8/100 WY, respectively [29]. Previous reports in southern Africa have demonstrated that HIV incidence rates are higher in women than in men [22]. In the present study, no incident HIV infections were identified in male participants. The gender disproportion has to be taken into consideration, since 76.8% of the participants were females.

Immunological and virological follow-up of the newly HIV infected participants showed a higher median CD4+ T-cell count (608 cells/mm³) and lower median HIV viral load (74,182 copies/mL) compared to findings by McKellar et al. [33]. In general, healthy women have higher CD4+ T-cell counts compared to men [34,35,36]. In our cohort, at baseline, the median CD4+ T-cell count was 824 cells/mm³ (IQR 434–1479) in a subset of 155 female participants, and 713 cells/mm³ (IQR 357–1155) in a subset of 102 male participants [35]. The four month interval between study visits could have led to a late diagnosis of acute HIV infection, suggesting that immunological recovery was already taking place.

Our study has demonstrated an overall prevalence of HbsAg of 12.2% which is higher than the previously reported prevalence in the country. In Mozambique previous studies in blood donors have reported a HBsAg prevalence of 10.6% [8], 9.3% [9] and 6.01% [37]. Reports in other African countries have shown prevalence of HBV infection as high as 15.5% [38] and 22% [39]. Transmission of HBV in Africa mainly occurs during childhood [6]. The majority of these youths never received Hepatitis B vaccinations. In Mozambique, Hepatitis B vaccination was introduced in the Expanded Program on Immunization in 2001 [40] and is available, free of charge, for infants. Vaccination boosts are not part of the national vaccination program. Therefore, transmission during childhood cannot be excluded. An important step in the epidemiology of Hepatitis B was the understanding that HBV can also be transmitted by sexual contact [6]. In the present study, 35% of youths reported to not have used a condom at the time of the most recent sexual intercourse. Such behavior may contribute to the higher prevalence of HBV infections in this cohort when compared to blood donors [9,37]. Our findings have showed that men were more affected than women (15.9% vs 11.1%), corroborating with previous findings in blood donors [9]. Co-infection with hepatitis B virus is common among HIV-infected individuals [41,42]. In our study we found that 11.3% of HIV-infected individuals were also infected by HBV. These findings are concordant with other results in sub-Saharan Africa, [41,43] indicating that HIV-infected patients could potentially be infected by HBV and could therefore benefit from HBV screening and vaccination. Our study demonstrates that youths are severely affected by HBV infection, and therefore, vaccination should be considered.
in early ages (to those who were not vaccinated after birth) and boosts given timely (to those who received primary vaccination).

Our results have shown a lower seroprevalence (0.36%) of syphilis compared to previous studies in the same population (2.3%) [12] and with the national rates in pregnant women in 2011 (2.2%) [20]. WHO reports have shown that in 2010, the prevalence of syphilis among women attending antenatal services in Mozambique was 5.7% [44]. Neighboring countries have also reported a high prevalence [45]. Syphilis screening has been practiced in Mozambique since 1978, but it was only in 1995 that it became a key element of the national health plan [46] particularly focusing on pregnant women. The northern region of Mozambique has a higher prevalence of syphilis compared to the south (8.2% vs 1.2%). The proximity to countries with high prevalence of syphilis in the northern part of Mozambique could have contributed to the higher prevalence in this region. Antenatal epidemiological rounds in Mozambique [11,47] have demonstrated that syphilis rates decrease in women with higher education, which supports the findings in our study (45% of the study population had higher educational degree, above secondary level). Additionally we found that all participants with a reactive syphilis test had other sexually transmitted viral infections such as HIV and/or HBV infections, suggesting that the route of transmission was most probably the same and that infection with one STI is a risk for acquisition of other venereal infections [5].

This study was conducted at a youth clinic in Maputo Central Hospital, the main referral hospital in the country. Educational activities and HIV counselling and testing at this site are very well structured and consistently available. The site is centrally located and easily accessible. Many secondary schools and universities are located in the same district area, thus facilitating access to the site. In Mozambique, sexual educational activities also take place at schools and to some extent at universities. Taking into account that almost all participants in this study were students from the surrounding areas, with potential access to information and education in regards to STIs prevention, it is very likely that this cohort with a lower HIV incidence is not representative of youth in Maputo.

One of the limitations of our study was that our cohort was female dominant, with more than 2/3 of the study population being females. This is a reflection of a gender imbalance in the demand for sexual and reproductive health services. This fact could have contributed to the total failure to detect seroconversion in male participants. Since only antibody tests were used for screening, and not the fourth generation combined assays for HIV antibodies and antigen, it cannot be excluded that a few recently infected cases in diagnostic window phase were missed at the last study visit. Incentives are commonly used in population-based cohort studies, and have been shown to be effective in improving retention rates [48,49]. Our retention rate, although relatively stable, was lower on the 3rd study visit. The fact that no monetary compensation was given to the participants may have influenced the rates of attendance since the study population was primarily students with probable economic restrictions.

**Conclusion**

Our study characterized a cohort of youths at an outpatient youth clinic specialized in provision of sexual and reproductive health services in Maputo. With the relatively low incidence of HIV, no significant association of risk behavior with HIV acquisition, and with a stable and adequate retention rate we can conclude that the cohort is suitable for possible recruitment into phase I/II HIV vaccine trials and other biomedical intervention studies. This study confirms the endemicity of HBV infections in Maputo, Mozambique suggesting that vaccination campaigns targeting youths should have an impact on the reduction of new HBV infections.
Supporting Information

S1 Table. Baseline socio-demographic and behavioral characteristics and HIV prevalence in male participants.

S2 Table. Baseline socio-demographic and behavioral characteristics and HIV prevalence in female participants.

Acknowledgments

The authors gratefully acknowledge the study participants, the dedicated staff at the youth clinic in Maputo Central Hospital, the staff at the Instituto Nacional de Saúde laboratories and all the collaborators from the Public Health Agency of Sweden.

Author Contributions

Conceived and designed the experiments: EOV NT IVJ NO ES GB CN SA CDS NBB OA. Performed the experiments: EOV NT EM EG CA NBB BM NS NI CDS NO IVJ. Analyzed the data: EOV OA ES GB CN SA IVJ NO NS BM NBB. Wrote the paper: EOV NT OA CDS NBB ES GB CN SA IVJ NO.

References

1. UNAIDS JUNPoHA. Global report: UNAIDS report on the global AIDS epidemic 2013. Switzerland. 2013; p2–7.
2. UNAIDS. AIDSinfo Epidemiological Status. 2014. Available: http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/content/25/08/2014.
3. Instituto Nacional de Saúde (INS) InDesI, ICF Macro. Inquérito Nacional de Prevalência, Riscos Comportamentais e Informação sobre o HIV e SIDA em Moçambique 2009. Maputo, Mozambique. 2010; p151–181.
4. UNAIDS. Core Epidemiology Slides. Available: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/201309_epi_core_en.pdf/content/30/08/2014.
5. Carpenter LM, Kamali A, Payne M, Kiwuuwa S, Kintu P, Nakiyingi J, et al. Independent effects of reported sexually transmitted infections and sexual behavior on HIV-1 prevalence among adult women, men, and teenagers in rural Uganda. J Acquir Immune Defic Syndr. 2002; 29: 174–180. PMID: 11832688
6. World Health Organization. Weekly epidemiological record. Switzerland. 2009; p405–420.
7. J.B. Tapko BT, Luís G. Sambo. Status of blood safety in the WHO African region, report of the 2010 survey. Republic of Congo. 2014.p2.
8. Stokx J, Gillet P, De Weggheleire A, Casas EC, Maendaenda R, Beulane AJ, et al. Seroprevalence of transfusion-transmissible infections and evaluation of the pre-donation screening performance at the Provincial Hospital of Tete, Mozambique. BMC Infect Dis. 2011; 11: 141. doi:10.1186/1471-2334-11-141 PMID: 21605363
9. Cunha L, Piouzeau C, Ingrand P, Gudo JP, Ingrand I, Mondiane J, et al. Use of replacement blood donors to study the epidemiology of major blood-borne viruses in the general population of Maputo, Mozambique. J Med Virol. 2007; 79: 1832–1840. PMID: 17935167
10. Newman L, Kamb M, Hawkes S, Gomez G, Say L, Seuc A, et al. Global estimates of syphilis in pregnancy and associated adverse outcomes: analysis of multinational antenatal surveillance data. PLoS Med. 2013; 10: e1001396. doi: 10.1371/journal.pmed.1001396 PMID: 23468598
11. Instituto Nacional de Saúde (INS) IndEsti, Grupo Técnico Multisectorial de Combate ao HIV/SIDA (GTM). Vigilância Epidemiológica do HIV e seu Impacto Demográfico em Moçambique: Actualização, Ronda 2009. Maputo, Mozambique. 2011; p36–40.
12. Melo J, Folgosa E, Manjate D, Osman N, Francois I, Temmerman M, et al. Low prevalence of HIV and other sexually transmitted infections in young women attending a youth counselling service in Maputo,
13. Ministério da Saúde. Estratégia Nacional de Comunicação para o Combate ao HIV/SIDA: Maputo, Mozambique. 2006; p6–22.

14. Esparza J. An HIV vaccine: how and when? Bull World Health Organ. 2001; 79: 1133–1137. PMID: 11799445

15. Ghys PD, Kufa E, George MV. Measuring trends in prevalence and incidence of HIV infection in countries with generalised epidemics. Sex Transm Infect. 2006; 82 Suppl 1: i52–i56. PMID: 16581761

16. Braunstein SL, van de Wijgert JH, Nash D. HIV incidence in sub-Saharan Africa: a review of available data with implications for surveillance and prevention planning. AIDS Rev. 2009; 11: 140–156. PMID: 19654856

17. Instituto Nacional de Estatística. Mulheres e Homens em Moçambique, Indicadores Seleccionados de Género Mozambique. Maputo, Mozambique. 2011; p15–17.

18. Ministério da Saúde. Guia Estratégico-Operacional para Implementação das Unidades de Aconselhamento e Testagem em Saúde (UATS). Maputo, Mozambique. 2008; p24–32.

19. Jani IV, Sabatier J, Vubil A, Subbarao S, Bila D, de Sousa A, et al. Evaluation of a high-throughput diagnostic system for detection of HIV-1 in dried blood spot samples from infants in Mozambique. J Clin Microbiol. 2012; 50: 1458–1460. doi: 10.1128/JCM.00107-12 PMID: 22278838

20. Ministério da Saúde, GTM. Ronda de Vigilância Epidemiológica do HIV e Sífilis em Moçambique, 2011, principais resultados. Maputo, Mozambique. 2013; p4–11.

21. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med. 2012; 13: 581–588. doi: 10.1111/j.1468-1293.2012.01018.x PMID: 22500780

22. Barnighausen T, Tanser F, Gqwede Z, Mbizana C, Herbst K, Newell ML. High HIV incidence in a community with high HIV prevalence in rural South Africa: findings from a prospective population-based study. AIDS. 2008; 22: 139–144. PMID: 18090402

23. Chege W, Pals SL, McLellan-Lemal E, Shinde S, Nyambura M, Otteno FO, et al. Baseline findings of an HIV incidence cohort study to prepare for future HIV prevention clinical trials in Kisumu, Kenya. J Infect Dev Ctries. 2012; 6: 870–880. doi: 10.3855/jidc.2636 PMID: 23276741

24. Bakari M, Lyamuya E, Mugusi F, Aris E, Chale S, Magao P, et al. The prevalence and incidence of HIV-1 infection and syphilis in a cohort of police officers in Dar es Salaam, Tanzania: a potential population for HIV vaccine trials. AIDS. 2000; 14: 313–320. PMID: 10716508

25. Gouws E, Stanecki KA, Lyerla R, Ghys PD. The epidemiology of HIV infection among young people aged 15–24 years in southern Africa. AIDS. 2008; 22 Suppl 4: S5–16. doi: 10.1097/01.aids.0000341773.86500.9d PMID: 19033755

26. Halietti TB, Lewis JJ, Lopman BA, Nyamukapa CA, Mushati P, Wambe M, et al. Age at first sex and HIV infection in rural Zimbabwe. Stud Fam Plann. 2007; 38: 1–10. PMID: 17385378

27. Heng BH, Goh KT, Chan R, Chew SK, Doraisingham S, Quek GH. Prevalence of hepatitis B virus (HBV) infection in Singapore men with sexually transmitted diseases and HIV infection: role of sexual transmission in a city state with intermediate HBV endemicity. J Epidemiol Community Health. 1995; 49: 309–313. PMID: 7629470

28. Aboodol Karim Q, Kharsany AB, Frohlich JA, Werner L, Mlotshwa M, Madlala BT, et al. HIV incidence in young girls in KwaZulu-Natal, South Africa—public health imperative for their inclusion in HIV biomedical intervention trials. AIDS Behav. 2012; 16: 1870–1876. PMID: 22618892

29. Nel A, Mabude Z, Smit J, Kotze P, Arbuckie D, Wu J, et al. HIV incidence remains high in KwaZulu-Natal, South Africa: evidence from three districts. PLoS One. 2012; 7: e35278. doi: 10.1371/journal.pone.0035278 PMID: 22536364

30. De Schacht C, Mabunda N, Ferreira OC, Ismael N, Calu N, Santos I, et al. High HIV incidence in the postpartum period sustains vertical transmission in settings with generalized epidemics: a cohort study in Southern Mozambique. J Int AIDS Soc. 2014; 17: 18808. doi: 10.7448/IAS.17.1.18808 PMID: 24629842

31. Feldblum PJ, Enosse S, Dube K, Arnaldo P, Muluana C, Banze R, et al. HIV Prevalence and Incidence in a Cohort of Women at Higher Risk for HIV Acquisition in Chokwe, Southern Mozambique. PLoS One. 2014; 9: e97547. doi: 10.1371/journal.pone.0097547 PMID: 24842811

32. Dube K, Zango A, van de Wijgert J, Meque I, Ferro JJ, Cumbe F, et al. HIV Incidence in a Cohort of Women at Higher Risk in Beira, Mozambique: Prospective Study 2009–2012. PLoS One. 2014; 9: e84979. doi: 10.1371/journal.pone.0084979 PMID: 24475035
33. McKellar MS, Cope AB, Gay CL, McGee KS, Kuruc JD, Kerkau MG, et al. Acute HIV-1 infection in the Southeastern United States: a cohort study. AIDS Res Hum Retroviruses. 2012; 29: 121–128. doi: 10.1089/AID.2012.0064 PMID: 22839749

34. Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Mwaengo DM, et al. Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in Western Kenya. PLoS One. 2011; 6: e21040. doi: 10.1371/journal.pone.0021040 PMID: 21713038

35. Tembe N, Joaquim O, Alfai E, Sitoe N, Viegas E, Macovela E, et al. Reference values for clinical laboratory parameters in young adults in maputo, mozambique. PLoS One. 2014; 9: e97391. doi: 10.1371/journal.pone.0097391 PMID: 24827458

36. Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, et al. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol. 2004; 11: 29–34. PMID: 14715541

37. Gudo ES, Abreu CM, Mussa T, Augusto Ado R, Otsuki K, Chambo E, et al. Serologic and molecular typing of human T-lymphotropic virus among blood donors in Maputo City, Mozambique. Transfusion. 2009; 49: 1146–1150. doi: 10.1111/j.1537-2995.2009.02100.x PMID: 19222818

38. Komas NP, Bai-Sepou S, Manirakiza A, Leal J, Bere A, Le Faou A. The prevalence of hepatitis B virus markers in a cohort of students in Bangui, Central African Republic. BMC Infect Dis. 2010; 10: 226. doi: 10.1186/1471-2334-10-226 PMID: 20670399

39. Abdoel Karim SS, Theijal R, Singh B. High prevalence of hepatitis B virus infection in rural black adults in Mseleni, South Africa. Am J Public Health. 1989; 79: 893–894. PMID: 2735483

40. Ministry of Health. Introduction to DPT-Hepatitis B Vaccine, Information for Health Workers. Maputo, Mozambique. 2001;p2.

41. Nyirenda M, Beadsworth MB, Stephany P, Hart CA, Hart IJ, Munthali C, et al. Prevalence of infection with hepatitis B and C virus and coinfection with HIV in medical inpatients in Malawi. J Infect. 2008; 57: 72–77. doi: 10.1016/j.jinf.2008.05.004 PMID: 18555534

42. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology. 2009; 49: S138–145. doi: 10.1002/hep.22883 PMID: 19399813

43. Negero A, Sisay Z, Medhin G. Prevalence of Hepatitis B surface antigen (HBsAg) among visitors of Shashemene General Hospital voluntary counseling and testing center. BMC Res Notes. 2011; 4: 35. doi: 10.1186/1756-0500-4-35 PMID: 21303563

44. World Health Organization. Investment case for eliminating mother-to-child transmission of syphilis, Promoting better maternal and child health and stronger health systems. Geneva, Switzerland. 2012; p9.

45. Kumogola Y, Slaymaker E, Zaba B, Mnonga R, Challenga J, et al. Trends in HIV & syphilis prevalence and correlates of HIV infection: results from cross-sectional surveys among women attending ante-natal clinics in Northern Tanzania. BMC Public Health. 2010; 10: 553. doi: 10.1186/1471-2458-10-553 PMID: 20836872

46. Gloyd S, Montoya P, Floriano F, Chadreve MC, Pfeiffer J, Gimbel-Sherr K. Scaling up antenatal syphilis screening in Mozambique: transforming policy to action. Sex Transm Dis. 2007; 34: S31–36. PMID: 17592388

47. Ministério da Saúde, GTM. Ronda de Vigilância Epidemiológica do HIV de 2007. Maputo, Mozambique. 2008; p33–35.

48. Booker CL, Harding S, Benzeval M. A systematic review of the effect of retention methods in population-based cohort studies. BMC Public Health. 2011; 11: 249. doi: 10.1186/1471-2458-11-249 PMID: 21504610

49. Doody MM, Sigurdson AS, Kampa D, Chimes K, Alexander BH, Ron E, et al. Randomized trial of financial incentives and delivery methods for improving response to a mailed questionnaire. Am J Epidemiol. 2003; 157: 643–651. PMID: 12672684