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HIV incidence and its associated factors among young adults with multiple sexual partners in Maputo, Mozambique: a vaccine preparedness study

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Abstract

Introduction Sub-Saharan Africa has a high burden of HIV, particularly among female sex workers (FSW) and men who have sex with men (MSM). Future clinical trials to evaluate vaccines and other interventions to prevent HIV will need to enroll populations with high HIV incidence. We conducted an observational study of HIV incidence among men and women with multiple sexual partners—including MSM and FSW—in Maputo, Mozambique, in order to prepare the country to conduct future efficacy trials of candidate HIV vaccines and other HIV prevention products.

Methods We conducted a prospective observational HIV incidence study in Maputo, Mozambique, that enrolled adults aged 18–35 years, without HIV, who had two or more sexual partners in the preceding three months. Recruitment strategies prioritized participation of MSM and FSW. Participants were followed for 24 months with HIV-1 testing every 3 months and staff-administered behavioral questionnaires every 6 months. Cox proportional hazard modeling was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for factors potentially associated with HIV acquisition.

Results From January 2014 to October 2017, 505 adults without HIV were enrolled with median age of 21 years (interquartile range:19–24); 41% were female and 82% were single. There were 19 HIV seroconversions (10 female and 9 male) during 943 person-years (PY) of observation (overall HIV incidence 2.02/100PY; 95%CI 1.21–3.15). The highest HIV incidence was observed among sex workers (2.08/100PY; 95%CI 0.25–7.52) and MSM (19.18/100PY; 95%CI 3.96–56.06). Increased hazard of incident HIV was observed among participants who were MSM (HR = 27.95, 95%CI 4.39–117.94), $p = 0.0004$), reported three or more sexual partners at enrollment (HR = 7.39, 95%CI 1.64–33.25, $p = 0.009$), and indicated ever having a sexual partner living with HIV (HR = 9.64, 95%CI 2.23–41.71, $p = 0.002$).

Conclusion Our findings may inform inclusion criteria for upcoming clinical trials of HIV prevention interventions, including vaccine candidates, which may prioritize enrollment of MSM, people with more than three sexual partners,

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and people with sexual partners who are living with HIV. These same populations are in need of further intervention to reduce HIV incidence.

Keywords Acquired immunodeficiency syndrome, Africa South of the Sahara, Risk factors, Sexual and gender minorities, Sexual behavior key populations

Introduction

Worldwide, around 38 million people were living with HIV in 2021, of whom about 75% were estimated to be on antiretroviral therapy (ART). Although HIV incidence had decreased by 32% since 2010, there were still 1.5 million incident cases in 2021, with about 45% occurring in sub-Saharan Africa [1]. A safe and globally effective HIV vaccine would be a valuable tool in the armamentarium to prevent HIV acquisition but has, thus far, been elusive [2].

In sub-Saharan Africa, women and girls are particularly vulnerable to HIV, having accounted for 63% of all new cases in 2021, with incidence rates that are three times higher among young women aged 15–24 years as compared to males of the same age [3]. Key populations (people who inject drugs, sex workers, transgender people, prisoners, and men who have sex with men) and their partners contributed to 46% of new HIV cases in the region in 2021 [3]. Recent data suggest that Mozambique had the second highest number of new HIV cases among countries in eastern and southern Africa [4]. As in other parts of sub-Saharan Africa, Mozambique has scaled up HIV prevention and care services dramatically over the last decade, with transition to a "test and treat" strategy and, later, "test and start." By 2023, over 2 million Mozambicans living with HIV were on ART, representing 99% of all people known to be living with HIV in the country. In 2015, the Determined, Resilient, Empowered, AIDS-Free, Mentored, and Safe (DREAMS) program was launched in Mozambique as an intervention to reduce HIV incidence among adolescent girls and young women and this was expanded to eight of the ten Mozambican provinces in 2020. Voluntary medical male circumcision was also scaled up during this time. HIV pre-exposure prophylaxis (PrEP) was introduced via pilot projects in 2018 and is now widely available.

Several studies have been conducted in Mozambique to help advance the development of a vaccine to prevent HIV. In a study to prepare for phase I/II testing of such a vaccine, young men and women attending a youth clinic were recruited without consideration of behavioral risk factors for HIV in Maputo city from 2009–1011; that study showed an HIV incidence of 1.4 cases per 100 person-years (PY) [5]. In a study of postpartum women in Chókwè, a district of Gaza province, overall HIV incidence was 3.2/100PY and was highest in participants with younger age, fewer children, higher education level of the woman's partner, and having had sex with someone other than one's primary partner [6]. In Beira, one of the largest cities in Mozambique, a cohort of women with sexual behaviors known to confer HIV risk showed an incidence of 6.5/100PY [7].

While there is some information on HIV incidence in specific groups from different regions of Mozambique, there is little information on HIV incidence and its correlates in populations that might be targeted for recruitment into future phase III preventive HIV vaccine trials. A Mozambican adult population could enhance HIV-1 subtype representation to support an extensive network of efficacy trials enrolling diverse target populations, including heterosexual populations with HIV risk and vulnerable, stigmatized, and marginalized key populations such as men who have sex with men (MSM) and female sex workers (FSW). Thus, we enrolled men and women with multiple sexual partners—including these key populations— into a longitudinal observational cohort study in Maputo, Mozambique, in order to assess HIV incidence and prepare the country to conduct phase III preventive HIV vaccine trials.

Methods

Study design

Between January 2014 and October 2017, a non-randomized, prospective, observational cohort study (RV363) was conducted at a research site located on the outskirts of Maputo, Mozambique, as previously described [8]. Briefly, the clinical research site, *Centro de Investigação e Treino em Saúde da Polana Caniço* (CISPOC), is affiliated with the Mozambican National Institute of Health (INS) which operates under the National Ministry of Health. The building houses a general hospital that provides primary outpatient health services to approximately 10% of the total population of Maputo City including antiretroviral therapy for adult patients living with HIV. Its mission is to generate and promote the incorporation of scientific and technological solutions to major health problems and conditions in Mozambique. Based on HIV prevalence and projected area incidence of at least 3 cases/100PY, the study targeted an enrollment of 500 people living without HIV (PLWOH) from among approximately 1200 projected screened individuals.

Adults aged 18–35 years from the surrounding community were recruited for enrollment. Eligibility was based on testing negative for HIV and reporting two or more sex partners in the last three months. For masking purposes, the study also enrolled a small group of people living with HIV (PLWH) who only differed from other participants in their HIV status [8].

Recruitment process

A community-based recruitment strategy was utilized. Community leaders and members of the research site's Community Advisory Board (CAB) were consulted for selection of recruitment focal points and recruiters. From November 2013 to November 2014, study staff distributed fliers on the streets, at night schools, and in bars and markets. Fliers indicated that a study was seeking 18- to 35-year-old male and female residents of Maputo city who were willing to enroll in a long-term study and be periodically tested for HIV. Recruitment was bolstered by FSW association-supported night-time activities at specific streets and night clubs. The local lesbian, gay, bisexual, and transgender (LGBT) association supported social events for the recruitment of MSM. A transgender individual was hired through the LGBT association as a recruiter for the study to target both MSM and FSW. Fliers were made available at the MSM and FSW associations as another recruitment strategy. People who showed interest in the study were given a referral coupon to present at the study site.

Study visits

When potential participants presented to the study site, they provided written informed consent and then were screened for study eligibility. Each enrolled participant was followed for up to 24 months without HIV and participants who acquired HIV were followed for at least 12 months from the date of seroconversion. Medical evaluations and education regarding HIV prevention were performed every 3 months. Behavioral risk assessment occurred every 6 months using staff-administered questionnaires. If HIV seroconversion was detected in-between behavioral collection visits, participants were asked to complete the behavioral questionnaire either at the visit when HIV was diagnosed or at an additional visit closely after in order to minimize participant burden. All participants received counselling and condoms for HIV prevention. Participants received 150 MZN (approximately 2.50 USD) as compensation for time and transportation at every visit.

Laboratory testing for HIV incidence

Incident HIV was the primary outcome of the study. At each 3-month visit, HIV testing was performed according to the Mozambican national guidelines with venous blood samples screened for HIV antibodies using the Alere Determine® HIV-1/2 (Alere, Japan) rapid test. Samples that were reactive on the Determine® rapid test were confirmed by Unigold HIV 1/2® (Trinity Biotech PLC, Ireland), a second rapid test. Indeterminate and discordant results were resolved by using a fourth-generation ELISA, Genscreen Ultra HIV Ag-Ab® (Biorad, France). In rare cases, HIV-1 RNA PCR or Western Blot could be used to confirm HIV status. Participants with a new HIV diagnosis were referred for care and treatment at a public health facility.

Sociodemographic and behavioral characteristics

Behavioral risk assessment occurred every 6 months using staff-administered questionnaires. Time-invariant variables based on self-reported participant demographic characteristics at screening for study eligibility included sex, age, highest education obtained, occupation, monthly income, marital status, age at sexual debut, circumcision status (for men only), and any history of STIs. Age was categorized into approximately brackets of similar size that were also intended to reflect general life-course changes (18–20. 21–24, 25–29, and 30–35 years). The household income variable was dichotomized to 'less than 5000 MZN' and 'more than 5000 MZN' in alignment with the first quartile of income (< 5000 MZN is approximately the bottom 20% of household incomes in Mozambique). Marital status was categorized as 'not married', 'separated/divorced', or 'married', with the married category combining monogamous, polygamous, and 'cohabitating' statuses. Age at sexual debut was categorized as < 15, 15–17, and 18–25 years. Time-variant variables included having a spouse or primary sexual partner, having a secondary sexual partner, condom use with primary partner, condom use with secondary partner, number of sexual partners in the last 3 months, partner older by 10 + years, partner younger by 10 + years, ever having had a partner with HIV, alcohol consumption before sex, self-assessed HIV risk, transactional sex, and sex of partner(s).

Statistical analysis

As participant behavioral data were collected in 6-month intervals, a modified 'last timepoint carried forwards' approach was implemented when determining the appropriate participant datapoint for time-varying analysis. Participants who completed the 24-month study visit (the final scheduled visit for participants without HIV seroconversion), the visit date for the 24-month visit was used for person-time calculations and responses to the behavioral questionnaire at that visit were retained.

For participants lost to follow-up, person-time was calculated using the last visit in which they had a completed HIV test result; behavioral data were imputed forwards from the last completed questionnaire if different from the date used for person-time calculations. Participants who seroconverted were associated with the visit in which their seroconversion was recorded for the purposes of calculating person-years contributed to the study. Similar to the handling of loss to follow-up, if the HIV seroconversion visit was also one in which behavioral data were collected, those values were used. In the present analysis, if the interval between the seroconversion visit and completion of the behavioral questionnaire was longer than 10 days, data from the previous completed questionnaire closest to the conversion date were imputed forwards due to the potential for recall bias and social desirability bias from responses which would include sizable amounts of time as PLWH.

Demographic and distributional response data from the screening visit were examined for differences between participants who did and did not experience HIV seroconversion using chi-square tests of association. Subsequent analyses incorporated both time-invariant and time-variant data.

Incidence rate was calculated as cases per 100 person-years, with confidence limits calculated using Byer's method to account for the relatively low case count. Bivariable and multivariable cox-proportional hazard models were constructed to assess the hazard ratios for each covariate. Covariates with at least one level with a univariable $p < 0.05$ were included in the multivariable models. Kaplan–Meier curves were generated for variables with a multivariable p-value < 0.01 but reflect the unadjusted hazard estimates.

Results

A total of 1150 participants were screened for eligibility for this observational cohort study, of whom 613 (53.3%) were found to be eligible and 534 participants were subsequently enrolled (Fig. 1). The majority of the non-eligible individuals (459/537, 85.5%) did not report two or more sexual partners in the three months preceding consent. Of the 534 enrolled, 505 participants were living without HIV (PLWOH) at the enrollment visit. A total of 441 participants who enrolled without HIV went on to complete the final study visit; early discontinuation was low, with the majority due to participant decision ($n = 43$, including 23 unable to keep visits, 18 moved from the area, 1 pregnancy, and 1 HIV seroconversion did not wish to continue), an additional 16 were lost to follow-up with no response to further contact, and 5 removed from the cohort due to an inability to adhere to the visit schedule, for a total of 64 participants in the cohort removed from observation prior to the target 24 months of follow-up.

Participant characteristics

The baseline socio-demographic, clinical, and behavioral characteristics of the 505 PLWOH are presented in Table 1. These included 242 females (47.9%). All females with available sexual partner data reported sex exclusively with male partners ($n = 235$). Of the 263 males, 11 (4.2%) were MSM. Participants had a median age of 21 (interquartile range [IQR] 19–24). The majority of the enrolled PLWOH had at least secondary level of education (92.3%), and 438 participants (86.7%) reported lower monthly income (defined as less than 5000 MZN). Sexual debut before the age of 15 years was reported by 74 participants (14.7%). Approximately one in five participants (20.5%) reported three or more sexual partners, and only 10 participants reported having a partner living with HIV. Fifty-seven participants (11.3%) reported exchanging sex with someone for goods, money or favors in the three months prior to study screening. Of all 505 enrolled PLWOH, 33 (6.6%) perceived themselves as being at high-risk of acquiring HIV. From the 261 males enrolled, there were 169 (64.0%) circumcised.

HIV incidence

During the 24 months of follow-up of the 505 PLWOH, a total of 19 participants (10 females and 9 males) acquired HIV (2.02 cases per 100 PY [95% CI: 1.21–3.15]). HIV incidence amongst MSM and FSW was 19.18 cases per 100 PY (95% CI: 3.96–56.06) and 2.08 cases per 100 PY (95% CI: 0.25–7.52), respectively. Figures 2 and 3 displays HIV incidence rates by quartiles of enrollment groups, disaggregated by sex and age categories (also see Supplemental Tables 1 and 2). The 3rd and 4th quartile of the enrollment represented the recruitment period when MSM and FSW were the main target population.

The number of male participants who enrolled dropped during these quartiles due to a high number of MSM who had positive HIV tests at the screening visit, which precluded their enrollment (Fig. 2). HIV incidence rate increased during study enrollment for males from 1.1 cases per 100 PY (95% CI: 0.1–4.1) in the 1st quartile to 3.2 cases per 100 PY (95% CI: 0.7–9.5) in the 4th quartile. The HIV incidence for females was higher during the 1st and 3rd quartiles (3.1 cases per 100 PY in both quartiles), and lower for the group of females enrolled in the 4th quartile with an HIV incidence of 1.4 cases per 100 PY (95% CI: 0.2–5.1).

More than three-quarters of the participants enrolled were in the age group of 18–24 years, which did not vary with the change in recruitment strategies. However, the

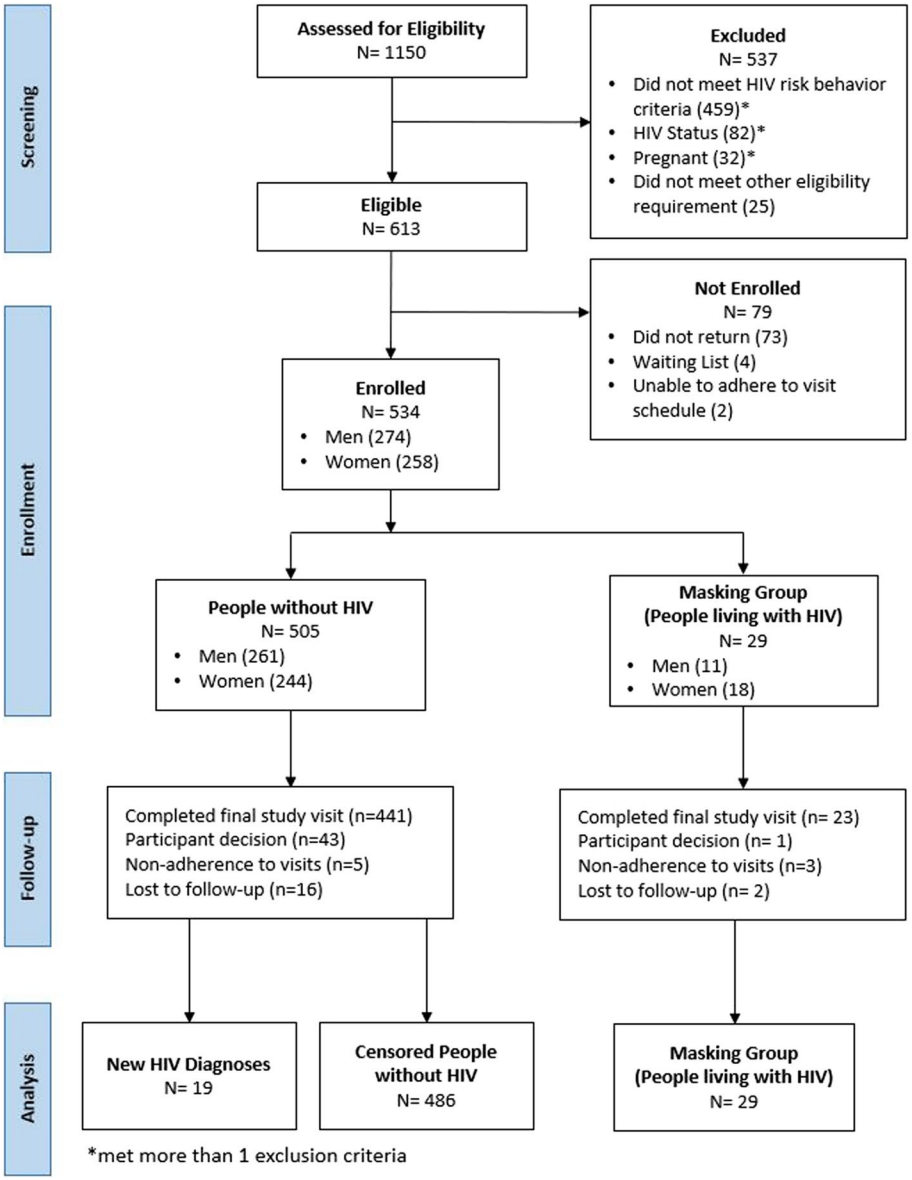

Fig. 1 RV363 screening, enrollment, and follow-up flowchart. "Participant Decision" refers to those who terminated from the study by their own discretion due to pregnancy, inability to keep visits, perceived social harms, not interested to continue after acquiring HIV, and moving from the area

HIV incidence rates were higher in the age group of 25–35 years with 3.4 cases per 100 PY (95% CI: 1.4–7.1) overall, having increased to an HIV incidence rate of 7.2 cases per 100 PY (95% CI: 0.9–26.0) in the last enrollment quartile.

Factors associated with HIV incidence

Table 2 describes the factors assessed for associations with HIV incidence. In the unadjusted models, a number of behavioral risk factors were significantly associated with HIV seroconversion. Having a secondary sexual partner as well as reporting 3 or more sexual partners in the last 3 months were associated with increased risk of HIV seroconversion. The risk of seroconversion during the study was associated with having had a partner older by 10 + years and also ever having a partner known to be living with HIV (compared to not knowing or not having had a partner living with HIV). Engaging in transactional sex and being MSM was associated with an increased risk of seroconversion during follow-up. The risk of HIV seroconversion during the study was associated with a self-perception of

Table 1 Sociodemographic and behavioral characteristics at enrollment, stratified by incident HIV during follow-up

Parameter	Category	Overall (*n* = 505)	Incident HIV, n (%)		*P*-value
			No (*n* = 486)	Yes (*n* = 19)	
Age	18 to 20 years	203(40.2)	197(40.5)	6(31.6)	0.320
	21 to 24 years	193(38.2)	187(38.5)	6(31.6)	
	25 to 29 years	75(14.9)	71(14.6)	4(21.1)	
	30 to 35 years	34(6.7)	31(6.4)	3(15.8)	
Partner-sex dyads	Male with only female partner(s)	252(50.6)	246(51.4)	6(31.6)	**< 0.001**
	Female with only male partner(s)	235(47.2)	225(47.0)	10(52.6)	
	Male with any male partner(s)	11(2.2)	8(1.7)	3(15.8)	
	Missing partner-sex dyad: n = 7 (all females with unspecified partner sex)				
Education	Completed primary or less	39(7.7)	36(7.4)	3(15.8)	0.179
	Some secondary or more	466(92.3)	450(92.6)	16(84.2)	
Monthly income	None	312(61.8)	301(61.9)	11(57.9)	0.924
	1 to 5000 MZN	126(25.0)	121(24.9)	5(26.3)	
	> 5000 MZN	67(13.3)	64(13.2)	3(15.8)	
Marital status	Single or widowed	415(82.3)	399(82.3)	16(84.2)	0.781
	Separated or divorced	73(14.5)	71(14.6)	2(10.5)	
	Married	16(3.2)	15(3.1)	1(5.3)	
Primary sexual partner	No primary partner	56(11.1)	56(11.5)	0(0.0)	0.117
	Has primary partner	449(88.9)	430(88.5)	19(100.0)	
Secondary sexual partner	No secondary partner	281(55.6)	272(56.0)	9(47.4)	0.459
	Has secondary partner	224(44.4)	214(44.0)	10(52.6)	
Number of sexual partners in last 3 months	0 partners	315(62.6)	303(62.6)	12(63.2)	0.991
	1–2 partners	85(16.9)	82(16.9)	3(15.8)	
	3–5 partners	103(20.5)	99(20.5)	4(21.1)	
Partner older by 10 years	No	483(95.6)	466(95.9)	17(89.5)	0.360
	Yes	21(4.2)	19(3.9)	2(10.5)	
	Missing/ Don't know 'Partner Older by 10 Years': n = 1				
Ever had partner with HIV	None	479(98.0)	461(98.1)	18(94.7)	0.312
	1 or 2	10(2.0)	9(1.9)	1(5.3)	
	Missing 'Ever had Partner with HIV': n = 16				
Age at sexual debut	< 15 years	74(14.7)	69(14.2)	5(26.3)	0.300
	15–17 years	270(53.5)	262(53.9)	8(42.1)	
	18–25 years	132(26.1)	126(25.9)	6(31.6)	
	Don't know	29(5.7)	29(6.0)	0(0.0)	
Self-assessed HIV risk	None	26(5.2)	25(5.2)	1(5.6)	0.514
	Some	437(88.1)	420(87.9)	17(94.4)	
	High	33(6.6)	33(6.9)	0(0.0)	
Transactional sex	No	448(88.7)	431(88.7)	17(89.5)	0.915
	Yes	57(11.3)	55(11.3)	2(10.5)	
Circumcised (males only)	No	95(36.0)	89(34.9)	6(66.7)	0.051
	Yes	169(64.0)	166(65.1)	3(33.3)	

All data are presented as n (column %). Comparisons were made between groups using Pearson's Chi-square test or, in cases with small cell sizes, Fisher's exact test

"high" risk of acquiring HIV compared to self-assessed "some" risk. The unadjusted models did not indicate any association between incidence of HIV and the sociodemographic variables age, partner-sex dyads, education, or monthly income.

In the multivariable analysis (Table 2), the hazard ratio for incident HIV was 7 times higher for participants with 3 sexual partners compared to having 1 or 2 partners (HR 7.4 [95% CI: 1.64–33.25], $p = 0.009$), and 15 times higher for those with 4 or more sexual partners compared to 1

Table 2 Associations of HIV incidence with sociodemographic and behavioral characteristics

Parameter	Category	HIV Incidence (Bivariable)		HIV Incidence (Multivariable)	
		Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value
Age	18 to 20 years	Reference			
	21 to 24 years	1.01(0.33–3.14)	0.981	-	-
	25 to 29 years	1.77(0.50–6.26)	0.378	-	-
	Age ≥ 30 years	2.86(0.72–11.44)	0.137	-	-
Partner-Sex dyads	Male with Only female partner(s)	Reference			
	Female with only male partner(s)	2.05(0.74–5.63)	0.166	1.95 (0.64–5.98)	0.240
	Male with any male partner(s)	**41.01(10.13–166.0)**	**< 0.001**	**27.95(4.39–177.94)**	**< 0.001**
Education	Completed primary or less	Reference			
	Some secondary or more	0.41(0.12–1.41)	0.157	-	-
Monthly Income	None	Reference			
	1 to 5,000 MZN	1.11(0.39–3.20)	0.843	-	-
	> 5,000 MZNmeticals	1.23(0.34–4.42)	0.747	-	-
Has Spouse or Primary Sexual Partner	No Primary Partner	Reference			
	Yes	2.40(0.32–17.99)	0.394	-	-
Has Secondary Sexual Partner	No Secondary Partner	Reference			
	Yes	**3.23(1.07–9.72)**	**0.037**	3.04 (0.93–9.98)	0.070
Number of sexual partners in last 3 months	1 to 2 partners	Reference			
	3 partners	**10.01(3.26–30.73)**	**< 0.001**	**7.39 (1.64–33.25)**	**0.009**
	4 + partners	**11.30(2.52–50.68)**	**0.002**	**15.23(1.80–129.20)**	**0.010**
Partner Older by 10 Years	No	Reference			
	Yes	**11.61(2.67–50.50)**	**0.001**	0.64 (0.09–4.66)	0.659
Ever had partner with HIV	No	Reference			
	Yes	10.66(3.10–36.67)	**< 0.001**	9.64 (2.23–41.71)	**0.002**
Self-assessed HIV risk	Some	Reference			
	None	1.48(0.20–11.21)	0.704	3.31 (0.42–26.22)	0.260
	High-risk	**4.84(1.40–16.72)**	**0.013**	2.00 (0.47–8.54)	0.350
Transactional sex	No	Reference			
	Yes	**13.04(1.64–103.6)**	**0.015**	2.35 (0.10–57.29)	0.601

Time-dependent variables reflect data collected closest to censor or seroconversion. Statistically significant hazard ratios and *p*-values ($p \leq 0.05$) are in bold

or 2 partners (HR 15.2 [95%CI: 1.80–129.20], $p=0.01$). Ever having had a partner with HIV continued to be significantly associated with the increased risk of incident HIV in the multivariable model (HR 9.6 [95% CI: 2.23–41.71], $p=0.0002$). Additionally, the risk of seroconversion was 28 times higher for MSM compared to men who did not report sex with men (HR 27.95 [95% CI: 4.39–177.94], $p=0.0004$). Having a secondary sexual partner, reporting a partner older than 10 + years, self-assessed HIV risk, and transactional sex did not remain significant after adjusting for other covariates. Figure 4 presents the Kaplan–Meier (KM) survival curves for independent variables with $p \leq 0.1$ in the adjusted model.

Discussion

This is amongst the first prospective studies that directly estimated HIV incidence among non-pregnant women and men in diverse communities affected by HIV (heterosexual, MSM and FSW) in Mozambique. This was conducted as a preparedness study for phase III HIV vaccine studies and lessons continue to apply to that context even as the landscape of HIV prevention and treatment evolves in Mozambique.

The findings document a moderately high incidence of HIV in this cohort, although empirical incidence data show a decline of new HIV acquisitions in sub-Saharan Africa [9]. Despite this, new HIV acquisition in sub-Saharan Africa remain concentrated and high in key populations, such as FSW and MSM; these groups were observed to have heightened HIV incidence in our study as well. An earlier HIV incidence study conducted among youth clinic attendees in Maputo, most of whom had multiple lifetime sexual partners, reported a lower HIV incidence of 1.49 per 100 PY [5]. The higher incidence rate in our cohort may reflect enrichment for key populations such as MSM and FSW. HIV incidence among FSW in our cohort was similar to that observed in previous studies of sex workers

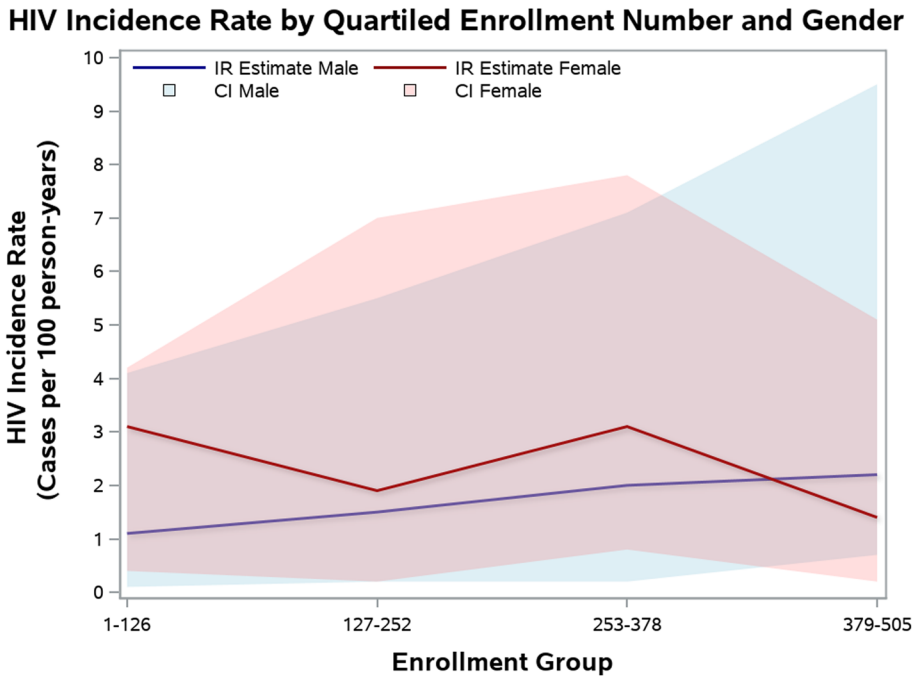

Fig. 2 HIV incidence rate by quartiled enrollment number and sex. Figure lines represent the trend in incidence across enrollment quartiles. Shaded regions depict the confidence intervals for incidence given cumulated incidence and person-time

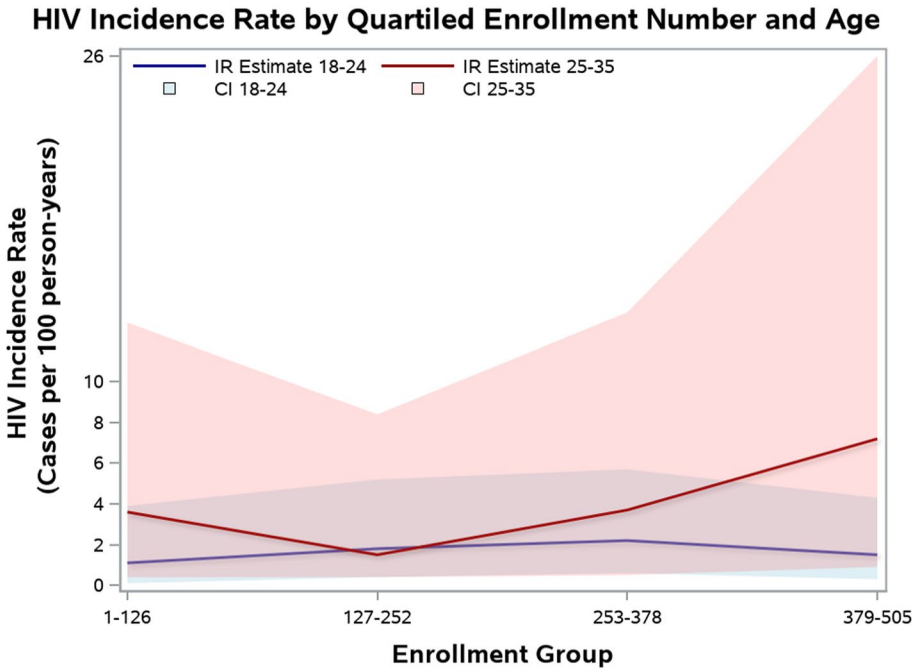

Fig. 3 HIV incidence by quartiled enrollment number and age. Figure lines represent the trend in incidence across enrollment quartiles. Shaded regions depict the confidence intervals for incidence given cumulated incidence and person-time

from sub-Saharan Africa [9]. HIV incidence among MSM in our cohort was much higher than the overall study cohort and higher than the incidence observed in previous studies conducted in Sub-Saharan Africa where HIV incidence among MSM and/or transgender women was no more than 16.0 per 100 PY [9–15].

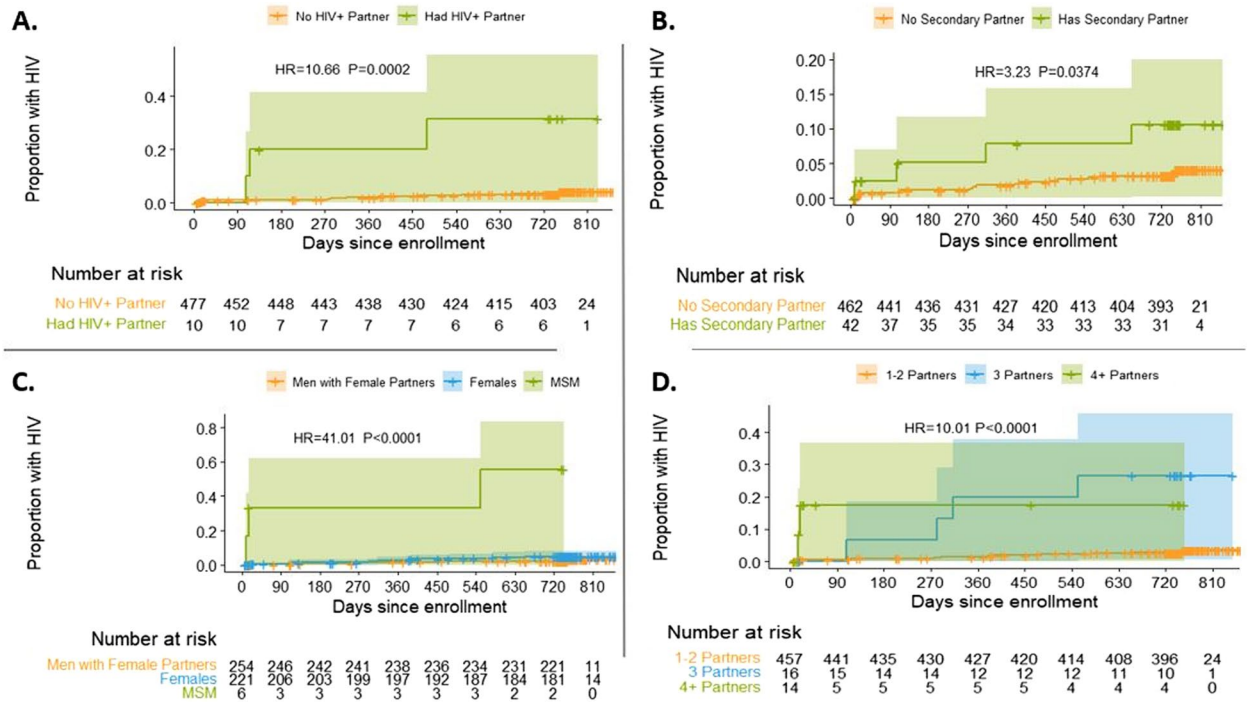

Fig. 4 Kaplan-Meier survival curves for incident HIV. Figure **A**-**D** represent the univariable Kaplan Meier curve for proportional hazard of HIV for a given covariate across the observation time. **A** depicts the participant reporting having had a PLWH in the prior 3 months; (**B**) depicts hazard associated with having a secondary sexual partner; (**C**) depicts hazard associated with partner diad; (**D**) depicts associated hazard with number of sexual partners in the prior 3 months

HIV incidence among females tended to be higher than among heterosexual males, which is consistent with the previous HIV incidence study conducted among youth in Maputo, Mozambique [5], and with the general HIV incidence epidemiology in Sub-Saharan Africa [9, 16]. Previous HIV incidence studies conducted in pregnant women [6] and in women with behavioral risk factors for HIV [7, 17] between 2008 and 2012 in Mozambique reported higher HIV incidence compared to our findings. This difference might be explained by the overall HIV preventive measures being implemented in Mozambique to reduce the number of new infections over time, including in young women. Study location difference might also explain the higher incidence of HIV in places with higher burden of HIV in Mozambique.

Age was not found to be a risk for HIV seroconversion, similar to findings from a previous study conducted in women in Mozambique [17]. Incidence rate by age and enrollment group were calculated (point-incidence and 95% CI), but differences were not formally tested in our study. HIV incidence among adults aged 25–35 years in each quartile was markedly higher compared to the HIV incidence among youths aged 18–24 years. Some caution should be taken with the age-specific incidence in this study as the overlapping confidence intervals between age groups indicate the difference may not rise to statistical significance. However, a previously conducted in South Africa and Kenya between 2006 and 2018 [18] also showed nearly twice the HIV incidence among older youths (20–24 years old) compared to younger ones (15–17 years), along with higher overall HIV incidence compared to our study. Given the general agreement between the studies, it may be that with additional participants in the 25–35 age group (which was nearly a third of the size of the younger group) would allow for tighter confidence intervals (which are notably wide in the 25–35 age group) and clarify any existing statistical difference.

Socioeconomic factors such as education were not associated with high-risk of HIV seroconversion. This finding was consistent with a previous HIV incidence study conducted in Mozambique including the finding from the low-risk cohort also from Maputo City, Mozambique [5, 17] but inconsistent with findings from other African countries [6, 10].

Being MSM, having multiple sexual partners and reporting a partner with HIV were associated with a high-risk of HIV seroconversion throughout the study. These findings show that the criteria to define high-risk groups should be adjusted to the local context in order to implement phase III HIV vaccine trials successfully in African countries.

It is important to note that this study was conducted before the adoption of "test and treat" and "test and start" strategies into HIV care programs in Mozambique and throughout sub-Saharan Africa. HIV care strategies have also evolved to include differentiated service delivery that is tailored to some of the key populations included in our study. The internal assessments of sociodemographic associations with relative risk of HIV acquisition in our study can meaningfully inform risk stratification regardless of current HIV treatment paradigms, but updated assessments are needed to understand how new treatment paradigms have affected HIV incidence. While some risks may have been attenuated since this study was conducted, it is unlikely that directions of association have changed, so those factors associated with highest risk of HIV acquisition in this cohort may still be used as meaningful criteria to identify study populations for future efficacy testing of HIV vaccine candidates and other HIV prevention interventions.

Limitations

This study has a number of limitations. Caution should be taken in the interpretation of point-estimates from the incidence analysis, particularly in the multivariable models. The low number of HIV seroconversions results in wide confidence intervals that add uncertainty to the true value of the point-incidence. However, we argue that even under a conservative approach of assuming the lower limits in the confidence interval rather than the point-estimate, the results still provide valuable insights into incidence dynamics captured by the cohort.

There were changes in recruitment strategies throughout the recruitment and enrolment period aiming to target more specific high-risk groups such as FSW and MSM. Using the same recruitment strategy to enroll heterosexuals and MSM proved to be a challenge, thus a limited number of MSM was enrolled in the study, which reduced the power of the findings for this specific group. Using a mixed- recruitment method, including respondent driven sampling, should be considered in future high-risk studies.

Self-reported behavioral data may be vulnerable to bias, including recall and social desirability biases. The study was implemented before the test-and-start era in Mozambique, which may not reflect the current dynamic of the HIV epidemic in the country. The study was conducted in the capital of Mozambique, which does not allow us to generalize the data to other locations or to other populations.

Despite the limitations, this study is one of the few sources of direct estimates of HIV incidence in different high-risk groups altogether, in a country with high burden of the disease. This allows to inform not only for future HIV vaccine trials but also for specific interventions in order to reduce the number of new HIV infections in high-risk groups and in the general young adult population. Although in Africa in general, MSM are not frequently engaged for research, this study provides crucial data that reinforces the need of providing PrEP for MSM and other high-risk groups, including discordant couples. Pilot projects to make PrEP available to key populations in Mozambique began in 2018 and future studies are needed to evaluate uptake and persistence of this important biomedical HIV prevention option.

Conclusion

In summary, the study demonstrated a moderately high incidence of HIV in the general community and high HIV incidence among MSM and among those with multiple sexual partners and partners known to be living with HIV in Maputo city, Mozambique. Existing HIV preventive measures should be reinforced among young females, MSM and discordant couples while new HIV prevention strategies, including HIV vaccine candidates, are potentially being evaluated in African countries, including in Mozambique.

Abbreviations

ART	Antiretroviral therapy
(F)SW	(Female) sex workers
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
KM	Kaplan-Meier
LGBT	Lesbian, Gay, Bisexual and/or Transgender
MSM	Men who have sex with men
PrEP	Pre-exposure Prophylaxis
PLWH	People living with HIV
PLWOH	People living without HIV
PY	Person-years

Supplementary Information

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Supplementary Material 1.

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Disclaimer

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Authors' contributions
NLM, MLR, CSP and IJ conceptualized the study design. IM, EV, RMC and VM contributed to the study implementation and data collection. AY, CN, MI, MM, QL, LAE, and TAC participated in data curation. AY performed data analysis and interpretation of results. NB, CN, SS, TM, ES, TAC and CSP provided project administration and oversight. IM, NB, AY, TAC, and CN prepared the draft manuscript. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials
To request a minimal data set, please contact the Data Coordinating and Analysis Center (DCAC) at PubRequest@hivresearch.org and indicate the RV363 study along with the name of the manuscript.

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the National Health Bioethics Committee of Mozambique (reference number 282/CNBS/13, IRB00002657) and by the Walter Reed Army Institute of Research Institutional Review Board (IRB00000794). Administrative approval was granted by the Ministry of Health of Mozambique. The protocol used for this study complied with International Conference on Harmonization Good Clinical Practice guidelines and was conducted in accordance with the principles described in the Nuremberg Code and the Belmont Report including all federal regulations regarding the protection of human participants as described in 32 CFR 219 and Army Regulation 70–25. All participants provided written informed consent before screening for study eligibility.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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