CONCISE COMMUNICATION

HIV acquisition prior to entry into formal sex work: inference from next-generation viral sequencing

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Objective: To infer the timing of HIV acquisition in relation to self-reported events in the sexual life course of adolescent girls and young women (AGYW) who self-identify as female sex workers (FSW) in Mombasa, Kenya.

Design: Next-generation viral sequencing of samples of AGYW living with HIV in the *Transitions* study, a cross-sectional bio-behavioural survey of AGYW aged 14–24 years in Mombasa, Kenya.

Method: Dried blood spot specimens were collected from study participants ($n = 37$, all FSW). A portion of the HIV *pol* gene was sequenced using an in-house next-generation sequencing assay for HIV drug resistance mutation genotyping. Estimated time since infection (ETI) was inferred using the HIV EVO web-based tool (https://hiv.biozentrum.unibas.ch/ETI/), and data on self-reported events were obtained from the survey.

Results: The median ETI among FSW was 3.4 (interquartile range = 1.7, 6.3) years, with a median ETI of 1.5 years prior to entry into formal sex work. We estimated that 74.1% (95% confidence interval = 53.7–88.9%) of participants living with HIV and who self-identified as FSW likely acquired HIV prior to self-identification as a sex worker.

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Conclusions: Findings suggest a large fraction of prevalent HIV infection among AGYW engaged in sex work stems from acquisition prior to entry into formal sex work. Current HIV prevention programs tailored for sex workers may miss key opportunities for HIV prevention as they are designed to reach women after entry into formal sex work, signaling a need for tailored programs to reach high-risk AGYW earlier on in their sexual life course.

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Keywords: adolescent girls, high-throughput nucleotide sequencing, HIV, sex work, viral genetics, young women

Introduction

Adolescent girls and young women (AGYW) experience disproportionate risks of HIV acquisition. In Kenya, AGYW constitute approximately 17% [1] of the population aged 15 years and older, but accounted for 30% of 34 610 new HIV infections in 2020 [2]. A combination of biological, structural, and network-level factors place AGYW at high risk of HIV acquisition, including vulnerabilities associated with transactional sex and formal sex work [3,4]. Data suggest programs designed and tailored for female sex workers (FSW) reach FSW several years after entry into formal sex work and have very low reach among AGYW who sell sex, with one study in Kenya finding only 13.7% of AGYW who sell sex had ever been contacted by a program [5]. Furthermore, programmatic and research cohort data suggest HIV prevalence is already high by the time FSW are reached by programmes, suggesting high rates of HIV acquisition prior to accessing tailored prevention programs. In Kenya, HIV prevalence among FSW (aged 18 years and older) upon enrollment in a programmatic and research cohort was already high at 30.3% during 2008–2011 [6]. Estimates of HIV incidence in the same cohort suggests a 2.3-fold higher risk during the early period of sex work [6].

Recent advances in next-generation sequencing (NGS) offer an opportunity to estimate time since HIV infection by quantifying viral diversity [7,8]. With more accurate estimates of time since infection (ETI), programmes could tailor and adapt HIV prevention services to data on the timing of risks in the sexual life course of individuals [7–9]. We used viral NGS to investigate the timing of HIV acquisition in relation to self-reported events in the early sexual life course of AGYW who self-identify as FSW in Mombasa, Kenya.

Methods

Study participants and sample collection

We used data from the *Transitions* study, a 2015 cross-sectional biobehavioural survey of AGYW aged 14–24 years in Mombasa, Kenya. Participants were recruited from sex work 'hotspots' as described by Cheuk *et al.* [10]. Following written informed consent, participants completed a questionnaire. Key dates in the sexual life course were self-reported by participants. All participants received point-of-contact HIV testing and counselling according to Kenya national guidelines [11]. Venous blood collected using standard phlebotomy [11] was used to prepare dried blood spot (DBS) specimens on Whatman 903 filter paper cards (GE Healthcare Life Sciences, Mississauga, Canada), which were air-dried for a minimum of 4 h. DBS specimens were stored at −80 °C in gas-impermeable bags containing desiccant pouches. Detailed data collection procedures are described by Becker *et al.* [12].

Serological testing

All DBS specimens were screened for HIV using the AVIOQ HIV Microelisa System (Avioq Inc., Durham, North Carolina, USA) according to the manufacturer's instructions at the National HIV and Retrovirology Laboratories (Public Health Agency of Canada, Winnipeg, Canada). HIV *pol* gene sequencing was attempted on all HIV-reactive DBS specimens.

HIV *pol* sequencing

A portion of the HIV *pol* gene (position 2074–3334 on HXB2, accession no. K03455) was sequenced using a routine, in-house, HIV drug resistance mutation genotyping assay [13,14]. Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, California, USA). A maximum of 96 libraries were pooled per sequencing run with a 5% spike-in of PhiX Control v3 library (Illumina). All steps of the library preparation were performed using an epMotion 5075t liquid handling workstation (Eppendorf, Hamburg, Germany). Sequencing was done on an Illumina MiSeq platform with the MiSeq Reagent Kit v2 (300-cycles; Illumina) according to the manufacturer's instructions. Although sequences from our study have not been deposited in public repositories (e.g. NCBI), they can be accessed upon request by contacting the corresponding author.

Estimation of estimates of time since infection

Illumina MiSeq reads were reference mapped to HIV HXB2-pol (accession K03455) using HyDRA Web (http://hydra.canada.ca), an open-source pipeline for analyzing next-generation sequence (NGS) data mainly for drug-resistant mutations. Here, we used it to generate intra-host single nucleotide variant (iSNV) calls. Within-host viral diversity was calculated using overall mean Hamming distance from the iSNV frequencies. The Hamming distance was then used to calculate the ETI in a web-based application, which explores NGS data as a biomarker to estimate infection dates (https://hiv.biozentrum.unibas.ch/ETI/) [7]. ETIs have a mean absolute error of approximately 1 year according to the application's developers.

Statistical analysis

We reported participant characteristics using descriptive statistics. To test for differences between FSW with and without HIV *pol* sequences from our dataset, we used the Fisher's exact and Mann–Whitney *U* tests for categorical and continuous variables, respectively. We estimated the overall ETI among self-identified FSW, and then stratified the ETI by three events to estimate the median difference between the ETI and the event, and to determine the proportion of acquisitions estimated to occur before/after each event: first sex, first transactional sex, and first self-identifying as a sex worker. Positive ETI values indicate HIV infection occurring after the date of the event, whereas negative ETI values indicate infection occurring prior to the event date. Analyses were conducted using SPSS Statistics, version 28 (IBM Corp., Armonk, New York, USA).

Ethics

Ethical approval was obtained from the Human Research Ethics Board at the University of Manitoba, Canada (HS16557); the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (P497/10/2017); and a research permit from the National Commission for Science, Technology and Innovation, Kenya.

Results

One thousand one hundred and ninety-three participants (91.8% of all study participants) consented to provide a DBS sample. Participants who did not consent to provide a DBS sample were more likely to be AGYW engaged in sex work and currently receiving formal education [11]. Serological testing identified 67 (5.6%) participants living with HIV, of whom 37 (55.2%) self-identified as FSW. Of these 37, 28 (75.7%) participants' samples were successfully sequenced. The observed ~25% sequencing failure is likely because of nucleic acid degradation during storage and/or low viral loads (<1000 copies/ml). Internal validation on DBS specimens has shown that the lower limit of detection of our in-house HIV drug resistance genotyping assay is approximately 1000 copies/ml (results not shown). Participant characteristics are described in Table 1, with little observed difference in participants with and without HIV *pol* sequences. The median age of participants was 21 years [interquartile range (IQR) = 19–23] at the time of the study. Median age at first sex was 16 years (IQR = 14–20). Participants reported engagement in sex work for a median of 2.5 years (IQR = 1–4).

Table 1. Sociodemographic characteristics of adolescent girls and young women n Mombasa, Kenya living with HIV who self-identify as female sex workers stratified according to HIV *pol* sequencing.

	FSW with successfully sequenced DBS samples		FSW with no sequence available	
	N = 28		*N* = 9	
	n	%	*n*	%
Age (years), median (IQR)	21 (19–23)		21 (21–23)	
Education level				
Never received formal education	3	10.7	0	0
Below secondary education	23	82.1	9	100
Full secondary education	2	7.1	0	0
Vocational education	0	0	0	0
Higher education, postgraduate	0	0	0	0
Ever pregnant	18	64.3	7	77.8
Ever married	3	10.7	0	0
Age at first sex (years)[a], median (IQR)	15 (14–17)		17 (15–18)	
Forced sex at first sex	4	14.3		
Age at first sex (years), forced sex, median (IQR)	16 (15–17)		NA	
Age at first sex (years), excluding forced sex, median (IQR)	15 (14–19)		17 (15–18)	
Active in sex work, years (IQR)	2.5 (1–4)		1 (1–4)	
Duration of sexual activity				
<4 years	8	28.6	2	22.2
≥4 years	17	60.7	6	66.7
Missing	3	10.7	1	11.1

[a]The *Transitions* study defined 'first sex' as either vaginal or anal intercourse [29]. Oral sex was not included. All participants (*n* = 37) reported vaginal sex at first sex. DBS, dried blood spot; FSW, female sex worker; IQR, interquartile range; NA, not available.

Estimated time of infection timeline

The median ETI for participants who self-identified as FSW was 3.4 [interquartile range (IQR) = 1.7–6.3] years. Figure 1 depicts the ETI stratified by years since first sex, first transactional sex, and first self-identifying as a sex worker. These events occurred sequentially among all participants. ETI was estimated to have occurred a median of 0.3 years after first sex (IQR = −3.1 to 4.2). The median difference between ETI and first transactional sex was −0.7 years (IQR = −5.1 to 1.3). The mean difference between ETI and first self-identifying as a sex worker was −1.5 years (IQR = −4.7 to 1.1), suggesting that FSW typically acquired HIV infections 1.5 years prior to self-identifying as a sex worker. Overall, 74.1% (95% confidence interval = 53.7–88.9%) of participants living with HIV and who self-identified as FSW were estimated to have acquired HIV prior to their engagement in formal sex work.

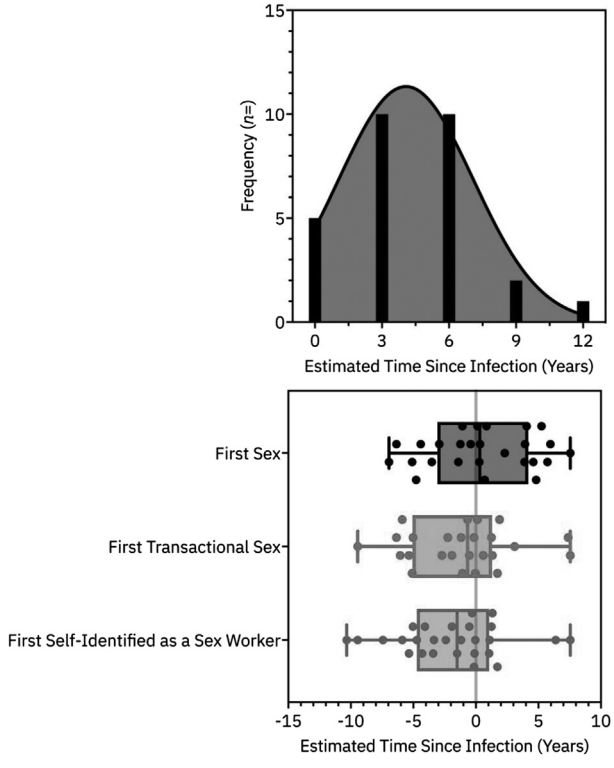

Fig. 1. Estimated time since HIV infection estimated from next-generation sequencing data in female sex workers in Mombasa, Kenya, compared with key self-reported events in their sexual life course. Reference-based mapping and variant calling was done against the HXB2 *pol* gene (loci 2253–5096, GenBank accession number K03455) using HyDRA Web (http://hydra.canada.ca). NGS reads were filtered by a minimum quality score of Q30 and 100 bp length. Variant calls were based on a minimum depth coverage of 100. Overall, the median NGS depth of coverage for each base was 73007 (IQR = 62512, 89890). ETI was estimated from NGS data using all codon positions as implemented by https://hiv.biozentrum.unibas.ch/ETI/. Top panel: distribution of all ETI values (years). Bottom panel: difference between ETI (years) and key points in the sexual life course of female sex workers is represented on the *y* axis. Key self-reported events are as follows: dates of first sex, first transactional sex, and the point at which they first self-identified as a sex worker. Values in the positive range demonstrate an ETI occurring after the date of the event, whereas values in the negative range demonstrate an ETI occurring prior to the event. ETI, Estimated time since infection; NGS, next-generation sequencing.

Discussion

Our findings suggest that, in the majority (74.1%) of AGYW who self-identify as FSW and are living with HIV, infection was acquired prior to engagement in formal sex work. On average, HIV acquisition occurred nearly 2 years prior to self-identifying as a sex worker.

To our knowledge, this study was the first study to use the online tool created by Puller *et al.* [7] to estimate time since infection in a real-world setting, and the first to use NGS data to describe the high occurrence of HIV acquisition among FSW prior to entering formal sex work. Due to the lack of similar studies, we are unable to compare our ETI estimates derived from NGS with other viral genomic data in the context of sex work. However, when compared with cross-sectional and cohort studies, our study corroborates the observation of high baseline HIV prevalence (12.3–43.9%) among FSW at HIV prevention program enrolment in Kenya, indicating HIV acquisition prior to program exposure [6,15]. The *Transitions* study also observed a high prevalence of HIV in AGYW who do not self-identify as FSW but are engaged in casual and transactional sex, further indicating that AGYW are highly vulnerable to HIV acquisition prior to entry into formal sex work [12].

Evidence suggests a complex interplay between biological, network/partnership, and structural factors that can shape HIV acquisition prior to sex work among AGYW currently engaged in sex work [16,17]. These include acquisition risks amplified in the context of age-disparate relationships [16–18], barriers to negotiating condom use [19,20], experiences of physical and sexual violence [21], barriers to accessing HIV prevention tools and services [5,22], and vertical transmission, although less likely in this context [23]. Several studies across countries in sub-Saharan Africa have identified differential exposure risks among young sex workers particularly in the first few years of sex work and potentially during a time when individuals may not self-identify as FSW [18,24,25]. Berthe *et al.* [25] found that informal sex workers, defined as nonprofessional or clandestine sex workers, were less likely to report routine condom use compared with those engaged in formal sex work. A similar study conducted in Central African Republic described a lower perception of HIV risk among informal sex workers, in addition to a

greater lack of knowledge regarding HIV [24]. Hensen *et al*. [22] found young women who sell sex but do not self-identify as FSW faced lower program access and awareness of HIV services in Zimbabwe compared with those who self-identified as FSW. Indeed, AGYW who do not self-identify as sex workers may be hesitant to engage with HIV prevention programs because of feelings of discomfort in participating alongside older female sex workers or unwillingness to be labelled as FSW before self-identifying as such [26].

A systematic barrier to addressing early acquisition risks stems from a policy and programmatic reality in many countries: informal sex workers and those engaged in transactional sex are rarely a focus population for HIV prevention programs [25]. Our findings suggest that HIV prevention programs designed and focused solely on reaching self-identified sex workers may be missing a key window of opportunity for HIV prevention before sex workers self-identify to themselves, their peers, or programs. FSW under the age of 18 years may be excluded from programming as many contexts have laws preventing those under 18 from accessing sex worker programs, which was the case in Kenya until 2018 [27,28]. In 2018, after the *Transitions* study was completed, Kenya implemented national guidelines for HIV and STI programming among young key populations, including AGYW who sell sex, as a result of evidence demonstrating that programs were not reaching AGYW who sell sex between the ages of 15 and 24 years [28]. Recommendations included adopting a venue-based approach at sex work hotspots, which has the potential to reach a large number of AGYW at increased vulnerability of HIV acquisition and undiagnosed AGYW living with HIV [11,28].

In summary, we estimated, using NGS, that a large fraction of prevalent HIV infection among AGYW engaged in sex work stems from acquisition prior to entry into formal sex work. Current HIV prevention programs tailored for sex workers may miss key opportunities for HIV prevention as they are designed to reach women after entry into formal sex work, signaling a need for tailored programs to reach high-risk AGYW earlier on in their sexual life course.

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B.N. and F.C. drafted and revised the manuscript. H.M., R.C., and C.D. acquired data. P.S., H.M., S.K., J.K., S.I., P.B., E.C., M.P., R.M., L.R.M., J.Bl., S.M., and M.B. contributed to the conception and design of the *Transitions* study. S.M. and M.B. critically revised and approved the final version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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