Dyspareunia, Signs of Epithelial Disruption, and HIV Serostatus in Female Sex Workers and Men Who Have Sex With Men in Nairobi: an Observational Study

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Abstract

**Background:** This study investigated HIV infection risk in men who have sex with men (MSM) and female sex workers (FSWs) by analysing signs of anal and vaginal epithelial trauma. It reconsidered the unique role of sexual abstinence for HIV seroconversion from a previous case-control analysis on FSWs in Nairobi who acquired HIV after previously fulfilling criteria of HIV resistance. The approach was based on a similarly unique role of intercourse frequency for anal dyspareunia. We considered confounding behavioural and individual HIV infection risks among the sex workers, who also assessed factors influencing discomfort and pain during sex from a subjective perspective.

**Methods:** At two key population facilities in Nairobi, 322 FSWs and 231 MSM provided data on HIV infection status, sexual dysfunction, intercourse frequency and abstinence behaviour. Additional data addressed sexual debut, relationship status, lubricants, foreplay, the number of sex partners, condom use, group and anonymous sex, vaginal births, intravaginal practices, sex toys, other sexually transmitted infections, alcohol and drug use. Statistical tests included t-tests for the equality of means for abstinence gaps and intercourse frequencies, the number of sex partners, vaginal births, and age of sexual debut. Non-parametric tests were used to compare HIV status and the ordinal variables of sexual behaviour, individual factors, dyspareunia and signs of trauma scores. Subjective assessments of variables for sexual dysfunction were given as percentages of the assessment options.

**Results:** Among FSWs, significant associations were found between HIV status and the longest abstinence gap for vaginal intercourse in the previous month, early sexual debut, foreplay, having anonymous partners, intravaginal practices, drugs and alcohol use, and all the sexual dysfunction or epithelial disruption signs. No significant association between HIV status and sexual dysfunction variables or sexual abstinence gaps could be found in the MSM sample. FSWs agreed that steady partnerships, regularity of intercourse, foreplay and lubrication or artificial lubricants alleviate discomfort and painful intercourse.

**Conclusions:** Dyspareunia and epithelial trauma signs were highly prevalent in FSWs and MSM, indicating considerable limitations to sexual health. Complaint levels were positively associated with HIV infection, suggesting that reducing epithelial disruption may be a novel HIV prevention approach.

**Background**

The World Health Organization emphasizes the need to focus on HIV prevention as the global number of people acquiring HIV has not declined enough to reach the set targets [1]. In the absence of an effective preventive HIV vaccine, innovative methods against HIV transmission seem all the more expedient. Sex workers and men who have sex with men (MSM) are among the key populations considered particularly vulnerable to HIV with regard to a high risk of infection, discrimination, and stigma [2].

Various behavioural and biological factors contributing to HIV spread have yielded a limited preventative potential. Firstly, while the presence of other sexually transmitted infections (STIs) is a crucial co-factor to
HIV transmission [3], attempts to reduce the spread of HIV infections by effectively treating other STIs have failed [4, 5]. Secondly, a review and meta-analysis of studies have shown that although there is a moderately significant association between intimate partner violence and HIV infection among women, there is significant heterogeneity of the association depending on the study region or country [6]. In addition, a systematic review of studies on intravaginal practices for "dry sex" or intravaginal cleansing found no conclusive evidence as to their causal role in the African HIV pandemic [7]. An eminent role of the biological transmission risk inherent in anal intercourse for the spread of HIV among MSM has previously been established [8]: Using powerful agent-based network simulation models, the study showed that a hypothetical reduction of the transmission risk of anal sex to that of vaginal sex could lead to an 80–98% reduction of HIV spread among MSM sexual networks even when MSM’s actual behavioural factors, circumcision, HIV testing and treatment levels are maintained in the model.

The study presented here examines HIV infection risk at the intersection of behaviour and biology in two key population samples. We investigated the association between dyspareunia, as well as direct signs of epithelial trauma, and HIV serostatus. We considered behavioural and other individual co-factors of both HIV infection and sexual dysfunction.

History of Research on Disproportionate HIV Spread, Natural Immunity and HIV Vaccines

An observational study [9] in a cohort of 424 initially HIV-1 seronegative female sex workers (FSWs) in Nairobi showed a decrease in HIV seroconversion with increasing exposure to HIV through sex work (12-fold for every weighted year of exposure, 100-fold cumulatively during the observed period; hazard ratio = 0.83, 95% CI [0.79, 0.88]; \( p < 0.001 \)). The sex workers' age, their sexual behaviour or the presence of other STIs were not associated with their persisting HIV-1 seronegativity. This and other subsequent observations [10] led researchers to suggest the possibility of the women being resistant to HIV infection, and to link the resistance to immunity resulting from HIV-specific cytotoxic T lymphocytes (CTL).

However, although at the end of the observation, 114 of these women had met criteria for HIV-1 resistance by remaining HIV-seronegative and PCR-negative for at least three years while continuing sex work, eleven of them seroconverted between 1996 and 2000 despite six of them having pre-existing HIV-1 specific CTL responses [11]. The seroconversion among these so-called "late seroconverters" was significantly associated with "having stopped sex work entirely for at least two months during the preceding year" (82% of seroconverters vs. 41% of controls; OR = 6.5, 95% CI [1.1–37.5]; \( p = 0.04 \)) and with a relative reduction of sex work by two or more clients per day (55% vs. 18%; OR = 5.4, 95% CI [1.1–26.9]; \( p = 0.04 \)).

Since the only significant association of the late seroconversion was the reduction or complete interruption of sex work according to the case-control analysis considering various HIV transmission risk factors, this finding was considered to be compatible with "loss or diminution of HIV-1-specific CTL in the absence of ongoing antigenic stimulation" by HIV [11]. A potential explanation for maintaining immunity
was the boosting of memory CTLs through continued antigen exposure, and HIV-1 resistance in the sex worker cohort was interpreted as an "immunologic state that is inducible given the correct antigenic stimulus" [11]. It was suggested that maintenance of HIV immune resistance required continuous antigenic priming, suggesting in turn that vaccination or vaccine boosters require persistent antigen presentation, and that it was not genetic but acquired.

**Rationale**

Our aim in this study was to reconsider the curious behavioural and temporal aspects of late HIV seroconversion. We thus investigated whether the regularity of sexual intercourse or shorter abstinence gaps were correlated with HIV serostatus among FSWs. This approach was motivated by theoretical considerations and a recent study on MSM's sexual health, leading to a double approach of studying several HIV risk factors, conjoined by the frequency of intercourse as a connecting link:

Theoretically, there was some incoherence between the hypothesis of continuous antigenic exposure as a prerequisite for maintaining HIV immunity in the sex worker cohort and the previous observation of a 12-fold decrease of HIV incidence for every weighted year of exposure to HIV through sex work between 1985 and 1994 [9]: HIV exposure may be assumed as merely occasional in the years before the general HIV epidemic in the Nairobi population. Rare exposure to HIV might have led to a loss or diminution of HIV-1-specific CTL comparable to that hypothesized for the late seroconverters who had taken a break from sex work. On condition that ongoing exposure to HIV-1 is a principle of HIV immunity [11], any lagged continuity of viral exposure in the initial phases of Kenyan HIV spread seems hard to align with a 100-fold protection against HIV transmission associated with starting sex work in 1985 as against 1994 [9]. At the end of the time span, continuous antigenic exposure would have seemed more likely than around 1985 because HIV prevalence had reached 10% in the general Kenyan population in 1994 [12]. The year had followed the incidence peak in 1992–1993, with a prevalence peak in 1995, whereas HIV spread was only beginning in 1985, and general HIV prevalence much lower [12].

We intended to align the two paradoxical epidemiological findings in Nairobi sex workers from the observation period between 1985 and 2000 by hypothesizing that higher frequency and regularity of sexual intercourse could be a principle of HIV immunity by virtue of a biological mechanism intertwined with behaviour: the reduction of epithelial trauma due to temporary genital dilation effects from sexual intercourse. On this account, the seeming protection against HIV acquisition by longer experience in sex work and the late HIV seroconversion after a reduction of sexual encounters seemed compatible with one another.

The empirical reason for studying intercourse regularity in two HIV key populations was a similarly unique role which the frequency of intercourse had turned out to play as a behavioural risk factor for anal dyspareunia in MSM [13]. Early "anodyspareunia" studies [14, 15] found a lack of lubrication, relaxation, or anal stimulation as well as anxiety as contributing to pain and discomfort. Vansintejan et al. studied ten risk factors [13]: age, the number of previous sex partners, the number of partners at a time, age of sexual debut, the frequency of sex with a partner, having a steady relationship, inhaled nitrite and condom
use, lubricant use, and foreplay. Statistical analysis using multivariate logistic regression showed that only age and the frequency of sex with a partner were correlated significantly with anodyspareunia (OR = 0.87, *p* < 0.0001; OR = 0.88, *p* = 0.018, respectively). Higher frequency of sex between MSM was associated with less pain.

Psychological contributing factors notwithstanding, painful intercourse for the recipient partner may be indicative of epithelial tissue being pressured, possibly causing traumatic breaches in the tissue. We connected the concept of such pressure to the accepted view that disruptions in the epithelial barrier facilitate viral penetration and increase the efficiency of HIV infection vaginally and anorectally [16]. Indicators of epithelial trauma have been observed in HIV key populations, sometimes involving a significant association with HIV seroconversion:

Three studies in South Africa examined the prevalence of genital bleeding: In the first [17], 36% of men and 28% of women experienced sexual contact involving blood in the previous three months. According to the second [18], more than 30% of both men and women reported engaging in sexual intercourse involving genital bleeding in the previous three months. The third [19] found that 31% of men and 26% of women had a lifetime history of engaging in sexual intercourse involving bleeding. A prospective study [20] found a statistically significant association between coital genital bleeding and HIV seroconversion.

A multivariate analysis of behavioural, psychological, and medical risk factors [21] showed that anal bleeding during sex affected a third of Mexican MSM at least sometimes, and that it was significantly associated with being HIV-positive. Associations between anorectal trauma, or indications of it, and HIV seropositivity had been previously found in one other cross-sectional study [22], where the multivariate relative odds for HIV seropositivity had been 7.72 given the highest level of rectal trauma indicators, and in two prospective studies [23, 24]. In a more recent study [25], 42% of MSM subjects in Senegal reported experiencing bleeding and discharge from the anus, and 22% having anal sores or tags. In South Africa, 60% of MSM subjects surveyed [26] reported experiencing some form of rectal trauma. Using conditional logistic regression models, rectal trauma with bleeding in the last 6 months was found to be a risk factor for HIV infection (OR = 2.947, 95% CI [1.308–6.638]) among MSM in Yunnan [27].

**Current Study**

Using the degrees of dyspareunia and physical symptoms such as bleeding as indicators of epithelial disruption, we investigated their association with HIV serostatus as a shortcut hypothesis for MSM and FSWs. With a questionnaire-based approach, we examined the influence of the frequency of sexual intercourse and of abstinence gaps on HIV serostatus. The basis for the study of sex frequency were the early results on HIV immunity among Nairobi sex workers [9, 11], indicating a link between temporary sexual abstinence and HIV transmission. Intercourse frequency served as a nexus between dyspareunia and HIV status for the shortcut relation to be investigated between these two variables because intercourse frequency, or at least abstinence gaps, had been significantly associated both with the late HIV seroconversions of Nairobi FSWs from 1996 to 2000 [11] and, more recently, with anodyspareunia among Belgian MSM [13]. The biological mechanism of dyspareunia as potentially increasing HIV
infection risk was its possible indication of epithelial anogenital trauma, an accepted HIV transmission risk [16]. We considered various confounding behavioural and individual factors to HIV infection in the sex worker sample and asked them to assess contributing and protective dyspareunia factors.

**Methods**

**Study Design**

Random sampling was done at specific key population facilities namely Bar Hostess for FSWs and Hoymas health facility for MSM. MSM and FSWs who had receptive sexual intercourse either within the previous month or whose prior sexual abstinence breaks did not exceed three months and were aged 18 years and above were included in the study, after giving their written informed consent. The interviews were conducted face-to-face with an interviewer filling out a questionnaire. Some FSWs were helped with translations.

**Measures**

Demographic information included gender, age, and education level. HIV serostatus was self-reported while the participants had previously been assessed, examined, and/or in follow-up treatment at the health facilities.

**Dyspareunia and Signs of Epithelial Disruption Score**

Questionnaires for FSWs included the last six questions of the Female Sexual Function Index (FSFI-19) [28]. The first three addressed satisfaction with (i) emotional closeness, (ii) with the sexual relationship(s), and (iii) the participant's satisfaction with overall sexual life in five grades ("very satisfied" to "very dissatisfied"). We grouped these three questions as a general sexual satisfaction cluster encompassing various psychological aspects of sexual well-being. The specific questions on arousal or desire, lubrication, and climax from the FSFI-19 were dropped for the sake of simplicity and since they were not directly related to signs of trauma or of epithelial tissue being directly pressured. The last three questions of the FSFI-19 directly addressed the frequency ("always or almost always" to "almost never or never") of discomfort and pain both (iv) during and (v) after vaginal penetration as well as (vi) the degree of discomfort or pain ("very high" to "very low or none at all"). We added two questions directly aiming at physical symptoms of epithelial disruption asking about (vii) the frequency (always to never) of any notice of blood on the genitals of the sexual partners not related to menstruation and (viii) on the frequency degree of vaginal itching or sensitivity during or after receptive intercourse.

Due to prior extensive research on anodyspareunia factors involving general relationship and relaxation factors [13] as well as to reduce complexity of the questionnaire, we dropped questions about foreplay and general relationship factors for MSM. MSM were asked three questions about the frequency of discomfort and pain both (i) during and (ii) after anal penetration as well as (iii) the degree of discomfort or pain. We added three questions asking about (iv) the frequency of itching and anal sensitivity, (v) the frequency of noticing blood that the participant believed came from his anus during or after receptive intercourse.
intercourse, and (vi) the frequency of the presence of blood on toilet paper in order to evaluate indicators of epithelial disruption more direct than pain and discomfort.

**Intercourse Frequency and Gaps of Abstinence**

Frequencies of intercourse and intervals of abstinence were asked in an identical fashion for the FSW sample and the MSM sample: Participants selected the number of receptive sexual intercourse events in the previous month. Use of sex-intended objects or sex toys the size of a penis were also counted as a receptive contact. Participants were additionally asked what the longest time gaps (in days) were between any instances of receptive vaginal (FSWs) or anal (MSM) intercourse (or comparable object insertion) in the previous month. To roughly gauge previous abstinence habits and phases, participants were asked to specify the longest abstinence gaps in their sexually active life; both including times of illness, a lack of potential partners, or other reasons for involuntary abstinence, and excluding involuntary reasons for abstinence such as partner availability, illness or disability affecting sexual life at the time.

**Sexual Behaviour and Individual Variables**

Based on the previous Belgian anodyspareunia study [13], questionnaires for FSWs included the age of sexual debut, relationship status and tendency of having steady partnerships, use of artificial lubricants, stimulation before intercourse, the number of different sex partners in a month, use of condoms, participation in group sex and having anonymous sex. We added some specific factors for women practising sex with men as the number of vaginal births and engaging in intravaginal practices (insertion of herbs, potions, powders or cleansing agents for dry and tight intercourse or vaginal cleansing). The interviewers also inquired about the rough duration of sexual intercourse, the use of sex toys, the presence of other STIs, and participation in sado-masochistic practices as further potential risk factors of HIV infection or epithelial disruption. As inhaled nitrite use is rather specific for MSM, we asked FSWs about several types of drug use including stimulants, inhaled nitrites, gamma-Hydroxybutyric acid, phencyclidin, and alcohol consumption in relation to sex.

**Subjective Estimates on Dyspareunia Factors**

In addition to behavioural and individual traits, we asked the FSWs to assess the impact of the following circumstances, habits and behaviour for increasing or decreasing discomfort and pain during sex from their own perspective: having a steady partner, higher number of sex partners, several partners at a time, higher age, longer duration of sex, increasing regularity or frequency of intercourse, stimulation or foreplay, lubrication or artificial lubricants, condom use, sex toy insertion before sex, and sex drugs or alcohol use. FSWs judged whether dyspareunia occurred less or more given the items, whether they made no difference, or abstained from judgement.

**Statistical Analyses**

Data were analysed using STATA 13 statistical software. Statistical tests included t-tests for the equality of means for abstinence gaps and intercourse frequencies, the number of sex partners, vaginal births, and age of sexual debut. Non-parametric tests were used to compare HIV status and the ordinal variables
of sexual behaviour, individual factors, dyspareunia and signs of trauma scores. Subjective assessments of the role of behavioural and other variables for sexual dysfunction were given as percentages of the four assessment options.

Results

Participants

A total of 322 FSWs with a mean age of 27.8 ± 6.9 (SD) years completed the questionnaire. See Table 1. Two hundred and three of them (63%) reported to be HIV-negative, 119 (37%) HIV-positive. Four FSWs were excluded from the final data analysis because two pairs had given identical answers during a translation of the questionnaire. Most sex workers had a secondary education level (39%), on average 1.7 ± 1.4 (SD) vaginal births and a sexual debut at 16.1 ± 2.8 years. Longest abstinence breaks in sexually active adult life for involuntary reasons were more than twice greater (68.9 ± 63.5 days) than voluntary abstinence gaps (25.5 ± 32.1 days).

A total of 231 MSM with mean age of 26.6 ± 5.5 years were interviewed, most of whom (33.8%) had a university level education. See Table 1. Self-reported HIV seropositivity was 43.7%, and the time difference was less pronounced between maximum voluntary abstinence (56.2 ± 80.4 days) and maximum involuntary abstinence (82.7 ± 97.2 days) from receptive intercourse or comparable object insertion in adult life than in the FSW sample.
Table 1
Descriptive Statistics: Population characteristics

|                           | MSM          |             | FSW          |             |
|---------------------------|--------------|-------------|--------------|-------------|
|                           | n  | %    | n  | %    |
| HIV Status: Negative      | 130 | 56.3 | 203 | 63.0 |
| Positive                  | 101 | 43.7 | 119 | 37.0 |
| Education Level: Primary  | 20  | 8.7  | 75  | 23.2 |
| Secondary                 | 67  | 29.0 | 126 | 39.0 |
| Tertiary                  | 66  | 28.6 | 86  | 26.6 |
| University                | 76  | 33.8 | 36  | 11.2 |
| Age Mean(SD)              | 231 | 26.6(5.5)| 322 | 27.8(6.9)|
| Min-Max                   |     | 18–45 |     | 17–50 |
| No. of times had receptive intercourse last month: | | | | |
| Mean(SD)                  | 230 | 5.3(5.0) | 321 | 19.3(8.4) |
| Min-Max                   |     | 0–25 |     | 0–31 |
| Longest time gaps between any instance of receptive intercourse (previous month): Mean(SD) | 225 | 9.8(7.9) | 321 | 5.6(5.0) |
| Min-Max                   |     | 0–31 |     | 0–30 |
| Longest time gaps between any instance of receptive intercourse (sexually active life): Mean(SD) | 231 | 82.7(97.2) | 322 | 68.9(63.5) |
| Min-Max                   |     | 2-730 |     | 2-365 |
| Longest time gaps between receptive intercourse for voluntary reasons: Mean(SD) | 229 | 56.2(80.4) | 322 | 25.5(32.1) |
| Min-Max                   |     | 2-700 |     | 1-365 |
| Age at first sex: Mean(SD) |     |       | 323 | 16.1(2.8) |
| Min-Max                   |     |       |     | 6–28 |
| No. of vaginal births: Mean(SD) |     |       | 322 | 1.7(1.4) |
| Min-Max                   |     |       |     | 0–8 |

Dyspareunia and Signs of Trauma Scores, Abstinence Gaps, and Other Behavioral and Medical Variables
Female Sex Workers

Forty-two percent of FSWs reported discomfort or pain during vaginal penetration "sometimes" in the previous four weeks. Six percent said it had occurred always or almost always during vaginal sex. Table 2 shows the frequency levels of discomfort or pain following penetration. The greatest proportion of sex workers (43%) reported a moderate pain and discomfort level. Noticing blood on the vagina or the partner's penis not related to menstruation was common; 44% of the sex workers said it occurred about half the time during or after intercourse, and itching, burning or soreness had been experienced by 71% of FSWs about half the time during or after intercourse (Table 2). Internal consistency for the total eight item dyspareunia and epithelial disruption sign score was acceptable with Cronbach's alpha = 0.72. Internal consistency was good for the three pain frequency and level questions (iv)-(vi) (Cronbach's alpha = 0.81), acceptable for the three emotional and sexual relationship satisfaction items (i)-(iii) (Cronbach's alpha = 0.73), while the two questions on direct bleeding signs and vaginal sensitivity (vii)-(viii) lacked internal consistency if separated from the total score (Spearman-Brown prophecy coefficient = 0.58).

Fifty-six percent of the FSWs were in a steady relationship at time of the study while most of them (55%) had had 'some’ steady relationships in life; 7% always had a steady relationship. Artificial lubricant use was moderately common, 34% of the FSWs sometimes using it, 18% using lubricants more than half the time for sex, and 27% never using them. Foreplay with stimulation of the vagina before intercourse was rather common, with 63% of the FSWs having foreplay at least half the time and only 14% never. Sex toy use and insertion before intercourse was rather rare, 225 (71%) of the FSWs reporting never using sex-intended objects before intercourse and 74% of the FSWs saying they never use them on themselves. Two hundred forty-six FSWs (77% of the sample) had at least one STI diagnosed previously, most of whom (41%) had had a diagnosis of exactly one STI (either syphilis, chlamydia, gonorrhoea, genital herpes, trichomoniasis, hepatitis C, genital or anal warts). Twenty-four FSWs had a diagnosis of STIs four or more times. Condoms were used at least half the time by 71% of the FSWs. Sadomasochistic sexual practices were rare: 91% of FSWs never engaged in them. Four percent of FSWs engaged in sadomasochistic practices involving blood rarely, 2% sometimes, and 1% regularly. Group sex appeared rather common as 47% of FSWs reported participating in it at least rarely. Having sex with anonymous partners was common (58% of FSWs having anonymous sex partners at least sometimes), as were dry sex practices involving vaginal insertion of herbs, powders or cleansing agents (69% of FSWs said they practised intravaginal substance insertion at least sometimes). Stimulant drug use (68% never) as well as inhaling nitrites (78% never) or gamma-Hydroxybutyric acid use (86% never) was rather rare. However, use of sedative drugs or analgesics was moderately common among FSWs, 60% of whom reported never using such drugs in relation to sex while 32% admitted to taking them at least sometimes. Alcohol use was common, 43% of the FSWs reporting regular drinking in relation to sex, and only 8% reporting never consuming alcohol when having sex.
Table 2
Discomfort or pain and signs of trauma scores among FSWs

| How often discomfort or pain during vaginal penetration was experienced in the past 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Almost always or always | 19 | 6 |
| Most times (more than half the time) | 38 | 12 |
| Sometimes (about half the time) | 132 | 42 |
| A few times (less than half the time) | 66 | 21 |
| Almost never or never | 62 | 20 |
| Total | 317 | 100 |

| How often discomfort or pain following vaginal penetration was experienced in the past 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Almost always or always | 24 | 8 |
| Most times (more than half the time) | 89 | 28 |
| Sometimes (about half the time) | 115 | 36 |
| A few times (less than half the time) | 73 | 23 |
| Almost never or never | 17 | 5 |
| Total | 318 | 100 |

| Rating of level (degree) of discomfort or pain during or following vaginal penetration in the past 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Very high | 16 | 5 |
| High | 82 | 26 |
| Moderate | 136 | 43 |
| Low | 57 | 18 |
| Very low or none at all | 27 | 8 |
| Total | 318 | 100 |

| Has noticed blood on vagina or partner's penis during vaginal intercourse or after vaginal sex that was not related to menstruation: | Respondents (n) | Proportion (%) |
|---|---|---|
| Never | 37 | 12 |
| Rarely (less than half the time) | 118 | 37 |
| Sometimes (about half the time) | 141 | 44 |
| Most times (more than half the time) | 20 | 6 |
| How often discomfort or pain during vaginal penetration was experienced in the past 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Always | 2 | 1 |
| Total | 318 | 100 |

| Has ever noticed vaginal itching, burning, or soreness during or after receptive vaginal intercourse: | Respondents (n) | Proportion (%) |
|---|---|---|
| Never | 14 | 4 |
| Rarely (less than half the time) | 41 | 13 |
| Sometimes (about half the time) | 225 | 71 |
| Most times (more than half the time) | 36 | 11 |
| Always | 2 | 1 |
| Total | 318 | 100 |

**Inferential Statistics**

An independent sample t-test established a significant relationship between HIV status and the longest time gap of sexual abstinence for vaginal intercourse or any similar vaginal insertion during the previous month ($p < 0.001$) but none with a participant’s number of vaginal births ($p = 0.458$), the number of times she had receptive vaginal intercourse in the last month ($p = 0.259$) and the longest involuntary ($p = 0.158$) or voluntary ($p = 0.198$) abstinence gap in their adult sex life. See Table 3. HIV seropositivity among the FSWs was $0.874 \ (0.814–0.939) \ (\text{COR (95\%\ C.I.)})$ times less likely with increased intervals between any instance of vaginal intercourse or comparable object insertion in the previous month (at 1% level of significance). There was also a significant relationship between HIV status and age at first sex with a man ($p = 0.041$) (Table 3).

Significant results for the relationship between the ordinal scale behavioural, medical and other individual variables and HIV status according to Mann-Whitney U tests were found for the following traits or behaviours (see Table 4): finger stimulation or massage of the vagina before intercourse ($p = 0.010$), sexual intercourse with anonymous partners ($p = 0.001$), dry sex practices ($p = 0.005$), inhaled nitrite use in relation to sex ($p < 0.001$), use of sedative drugs or pain killers in relation to sex ($p = 0.004$), using alcohol in relation to sex ($p = 0.002$), overall satisfaction with sexual relationship in the past four weeks ($p = 0.016$), pain and discomfort levels during or following vaginal penetration in the past four weeks ($p = 0.048$) and level of epithelial trauma signs as associated with bleeding during or after vaginal intercourse ($p < 0.001$). In binary logistic regression of HIV status against the ratings of significant factors identified at the bivariate level, odds ratios were established as follows:
Table 3

$t$-test for equality of means for HIV positive vs. negative FSW parity, sex frequency and abstinence gaps, sexual debut, and number of sex partners in a month

| Independent Samples Test | $t$-test for Equality of Means |
|--------------------------|--------------------------------|
|                          | $t$ | df  | p-value | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference |
|                          |     |     |         |                |                        |                                      |
|                          |     |     |         |                |                        | Lower                       | Upper                       |
| Number of vaginal births had: | -.743 | 255.816 | .458 | -.117 | .157 | - .427 | .193 |
| Number of times had receptive vaginal intercourse in the last month: | 1.131 | 254.536 | .259 | 1.080 | .955 | -.800 | 2.961 |
| Longest time gap (in days) between vaginal sex or any comparable insertion in the last month: | -4.397 | 315.368 | .000 | -2.082 | .474 | -3.014 | -1.151 |
| Longest time gap (in days) between vaginal sex or any comparable insertion in sexually active life in general: | 1.416 | 193.529 | .158 | 11.282 | 7.969 | -4.435 | 26.999 |
| Longest time gap (in days) between vaginal sex or any comparable insertion in sexually active life for voluntary reasons: | -1.290 | 290.335 | .198 | -4.559 | 3.535 | -11.516 | 2.398 |
| Age at first sex with a man: | -2.053 | 235.154 | .041 | -.660 | .321 | -1.293 | -.027 |
| Number of sex partners had for receptive vaginal intercourse in a month: | -1.948 | 294.018 | .052 | -1.521 | .781 | -3.057 | .016 |
Table 4
(a) Non-parametric tests for independent behavioural, medical and individual variables for HIV positive vs. negative FSWs and (b) binary logistic regression for significant relationships between HIV status and behavioural or individual variables (FSWs)

| (a) Independent Variables                                                                 | Mann-Whitney U | p-value |
|-------------------------------------------------------------------------------------------|---------------|---------|
| Uses a water or silicone-based lubricant for receptive vaginal intercourse:                | 10677.000     | 0.142   |
| Has finger stimulation or massage of the vagina before intercourse:                        | 9817.000      | 0.010   |
| Has sex toys, plugs, or dildos inserted before vaginal intercourse:                        | 10630.500     | 0.065   |
| Uses sex toys, plugs, or dildos on self:                                                  | 10993.000     | 0.184   |
| Has ever been diagnosed with Syphilis, Chlamydia, Gonorrhoea, Genital Herpes, Trichomoniasis, Hepatitis C or genital or anal warts/HPV: | 6982.500      | 0.468   |
| Uses condoms for vaginal intercourse:                                                     | 10767.000     | 0.164   |
| Engages in BDSM (bondage, discipline/dominination, sado-masochism) practices:             | 11204.500     | 0.132   |
| Engages in BDSM practices that involve blood:                                             | 11526.000     | 0.422   |
| Takes part in group sex:                                                                  | 11158.000     | 0.372   |
| Has sexual intercourse with anonymous partners:                                           | 9274.500      | 0.001   |
| Engages in dry sex practices or insert herbs, potions, powders or cleaning agents in vagina: | 9738.500      | 0.005   |
| Usual length of receptive vaginal intercourse:                                            | 229316.55     | 0.610   |
| Uses stimulants in relation to sexual intercourse:                                         | 11509.500     | 0.657   |
| Uses inhaled nitrites (poppers) in relation to sexual intercourse (rare):                  | 9674.000      | 0.000   |
| Uses GHB (liquid G) in relation to sexual intercourse:                                     | 11341.000     | 0.339   |
| Uses sedative drugs or pain killers in relation to sexual intercourse:                     | 9730.500      | 0.004   |
| Uses alcohol in relation to sexual intercourse:                                            | 9507.000      | 0.002   |
| Level of satisfaction with sexual relationship in the past 4 weeks:                        | 9924.500      | 0.016   |
| Level of pain and discomfort during or following vaginal penetration in the past 4 weeks: | 10200.500     | 0.048   |
| Level of trauma as associated with bleeding during or after vaginal intercourse:          | 8487.500      | 0.000   |

**Significant at 1% level of significance

*Significant at 5% level of significance
At either 1% or 5% levels of significance (see Table 4), age at first sex with a man higher by one year reduced the risk of HIV infection 0.915 (0.840–0.996) times (COR (95% C.I.)). Unit increase of the rating of the frequency (five degrees: never to always) of finger stimulation or massage of the vagina before intercourse decreased the risk of HIV infection 0.787 (0.648–0.955) times. Increased frequency of sex with anonymous partners decreased the risk of HIV infection 0.674 (0.531–0.856) times. Increased frequency of intravaginal practices increased the risk of HIV infection 1.419 (1.114–1.808) times. More use of sedative or analgesic drugs and of alcohol during or before sex increased the risk of HIV infection 1.385 (1.119–1.713) and 1.439 (1.101–1.880) times, respectively. As the FSWs’ level of satisfaction with their sexual relationship(s) in the past four weeks increased, their risk of HIV infection decreased 0.854 (0.754–0.969) times. As the level of pain and discomfort during or following vaginal penetration in the past four weeks decreased, their risk of HIV infection decreased 0.888 (0.800–0.986) times. As the level of trauma as associated with bleeding during or after vaginal intercourse increased, their risk of HIV infection increased 1.569 (1.271–1.937) times.

### (a) Independent Variables

| Variable                                                                 | Mann-Whitney U | p-value |
|-------------------------------------------------------------------------|----------------|---------|
| Age at first sex with a man (t-test see Table 3):                       |                |         |
| Has finger stimulation or massage of the vagina before intercourse:     | 0.787          | 0.648–0.955* |
| Has sexual intercourse with anonymous partners:                         | 0.674          | 0.531–0.856** |
| Engages in dry sex practices or insert herbs, potions, powders or cleaning agents in vagina: | 1.419          | 1.114–1.808** |
| Uses sedative drugs or pain killers in relation to sexual intercourse:  | 1.385          | 1.119–1.713** |
| Uses alcohol in relation to sexual intercourse:                         | 1.439          | 1.101–1.880** |
| Level of satisfaction with sexual relationship in the past 4 weeks:     | 0.854          | 0.754–0.969** |
| Level of pain and discomfort during or following penetration in the past 4 weeks: | 0.888          | 0.800–0.986* |
| Level of trauma as associated with bleeding during or after vaginal intercourse: | 1.569          | 1.271–1.937** |

**Significant at 1% level of significance

*Significant at 5% level of significance
trauma and bleeding signs during or after vaginal intercourse increased, their risk of HIV infection increased 1.569 (1.271–1.937) times.

**Subjective Assessment of Factors**

Eighty-one percent of FSWs agreed that steady partnerships lessen dyspareunia (Table 5). They were divided on whether having had more partners is of contributing or protective influence: Half (50%) of the FSWs judged discomfort and pain to decrease with more experience, yet more than a third of them (34%) found that it has no relevant effect on dyspareunia, and 10% believed sexual dysfunction worsens with further tries. Group sex or having several partners simultaneously seemed to increase sexual discomfort for 50% of sex workers. Yet 17% of the FSWs felt that several partners at a time reduce sexual dysfunction and another 20% said it made no difference. A slight absolute majority of 53% agreed that age did not play a role. Subjective judgements favouring a contributing (18%) and a protecting (14%) influence of maturing on dyspareunia roughly levelled each other out while 15% of participants claimed not to be in a position to compare the effect of age. More than two-thirds (69%) agreed that longer duration of vaginal intercourse leads to higher discomfort. The regularity of intercourse seemed to play a prominent role in reducing dyspareunia for participants. This was the second most unanimous assessment among the items: 74% of the FSWs agreed that higher regularity of intercourse alleviates sexual dysfunction. Foreplay or stimulation seemed to facilitate intercourse according to the unison of 73% of sex workers. Almost two-thirds (65%) agreed that lubrication or artificial lubricants ease difficulties. Participants seemed to confirm a lacking influence of condoms on dyspareunia. Sixty-one percent agreed that they make no difference to the dysfunction, and those participants (15%) saying condoms increase trouble were levelled out by an equal number (15%) saying condoms reduce trouble. The last two factors, sex toy and sex drug use, refer to practices that were uncommon among the FSW sample although alcohol played a central role for sexual encounters. Sex-intended object insertion was rare. Half of the FSWs abstained from judgement for these two items. Eighteen percent of FSWs said sex toys may ease trouble and 20% said they do not have a relevant effect. Almost each participant saying drugs facilitate sex (22%) had a counterpart in the sample saying drugs have no effect on pain and discomfort (20%), and eight percent of the sample agreed that drugs and alcohol worsen discomfort.
Table 5
Subjective assessment of factors to dyspareunia by FSWs

| Independent Variables                                                                 | Less (%) | No difference (%) | More (%) | Cannot Compare (%) |
|--------------------------------------------------------------------------------------|----------|-------------------|----------|--------------------|
| Feels sexual discomfort or pain during vaginal intercourse occurs less or more... with a steady partner than with casual sex partners: | 258 (81) | 43 (14)           | 9 (3)    | 8 (3)              |
| ...the more partners you already had receptive intercourse with:*                     | 158 (50) | 109 (34)          | 30 (9)   | 20 (6)             |
| ...with a higher number of partners you have sex with at a time (as in group sex, etc.):* | 53 (17)  | 63 (20)           | 159 (50) | 42 (13)            |
| ...with higher age:                                                                   | 43 (14)  | 170 (53)          | 58 (18)  | 47 (15)            |
| ...with longer duration of intercourse:                                              | 30 (9)   | 47 (15)           | 219 (69) | 22 (7)             |
| ...with higher regularity or frequency of vaginal intercourse and/or object use:      | 236 (74) | 35 (11)           | 37 (12)  | 10 (3)             |
| ...after foreplay or finger stimulation before intercourse:*                          | 231 (73) | 41 (13)           | 31 (10)  | 14 (4)             |
| ...with lubrication or lubes and oils:                                               | 208 (65) | 36 (11)           | 12 (4)   | 62 (19)            |
| ...with condoms:*                                                                    | 47 (15)  | 194 (61)          | 47 (15)  | 29 (9)             |
| ...after object insertion immediately before intercourse:*                           | 56 (18)  | 74 (23)           | 19 (6)   | 168 (53)           |
| ...with certain sex drugs such as GHB, alcohol, etc.:                                | 69 (22)  | 63 (20)           | 27 (8)   | 159 (50)           |

*n = 317

MSM

Prevalence of dyspareunia in MSM was high, with 195 (84%) experiencing at least some discomfort and pain in relation to anal intercourse. Sixty percent of them experienced pain during anal penetration at least sometimes, 17% always or almost always. Pain following anal penetration was experienced half the time by 33%, and most times by 20% of the MSM. Roughly a third of participants rated the degree of pain to be non-existent or low, another third as moderate, and another as high or very high. See Table 6. Seventy-seven percent of MSM complained about anal itching, burning or soreness at least half the time when having receptive sex. Traces of blood believed to be of anal origin were seen by 45% of MSM half
the time, and by 12% most times. Blood was present on the toilet paper in 43% of MSM half the time, in 13% most times. The overall internal consistency of the six item score of pain frequency, level, and of the direct epithelial disruption signs was good (Cronbach's alpha = 0.88).
### Table 6
Discomfort and pain as well as sensitivity or bleeding scores among MSM

| How often discomfort or pain during anal penetration by a partner was experienced in the last 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Did not attempt intercourse | 12 | 5 |
| Almost always or always | 39 | 17 |
| Most times (more than half the time) | 39 | 17 |
| Sometimes (about half the time) | 59 | 26 |
| A few times (less than half the time) | 52 | 23 |
| Almost never or never | 30 | 13 |
| Total | 231 | 100 |

| How often discomfort or pain following anal penetration by a partner was experienced in the last 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Did not attempt intercourse | 12 | 5 |
| Almost always or always | 17 | 7 |
| Most times (more than half the time) | 47 | 20 |
| Sometimes (about half the time) | 77 | 33 |
| A few times (less than half the time) | 53 | 23 |
| Almost never or never | 25 | 11 |
| Total | 231 | 100 |

| Rating level (degree) of discomfort or pain during or following anal penetration by a partner over the past 4 weeks: | Respondents (n) | Proportion (%) |
|---|---|---|
| Did not attempt intercourse | 11 | 5 |
| Very high | 11 | 5 |
| High | 50 | 22 |
| Moderate | 76 | 33 |
| Low | 55 | 24 |
| Very low or none at all | 27 | 12 |
| Total | 230 | 100 |

| Has ever noticed anal itching, burning, or soreness during or after receptive anal intercourse: | Respondents (n) | Proportion (%) |
|---|---|---|
| Never | 12 | 5 |
How often discomfort or pain during anal penetration by a partner was experienced in the last 4 weeks:

| Frequency                          | Respondents (n) | Proportion (%) |
|------------------------------------|-----------------|----------------|
| Rarely (less than half the time)   | 41              | 18             |
| Sometimes (about half the time)    | 142             | 61             |
| Most times (more than half the time) | 29              | 13             |
| Always                             | 7               | 3              |
| Total                              | 231             | 100            |

Has ever noticed blood believed to have come from anus during or after receptive anal intercourse:

| Frequency                          | Respondents (n) | Proportion (%) |
|------------------------------------|-----------------|----------------|
| Never                              | 14              | 6              |
| Rarely (less than half the time)   | 86              | 37             |
| Sometimes (about half the time)    | 103             | 45             |
| Most times (more than half the time) | 28              | 12             |
| Total                              | 231             | 100            |

Noticed blood on the toilet paper:

| Frequency                          | Respondents (n) | Proportion (%) |
|------------------------------------|-----------------|----------------|
| Never                              | 12              | 5              |
| Rarely (less than half the time)   | 90              | 39             |
| Sometimes (about half the time)    | 99              | 43             |
| Most times (more than half the time) | 30              | 13             |
| Total                              | 231             | 100            |

There were no significant differences between HIV-positive and HIV-negative MSM and anal sex frequencies during the previous month, their maximum abstinence gaps in the previous month, during adult life in general, nor for their maximum remembered voluntary abstinence interval (t-test: p = 0.439, p = 0.878, p = 0.259, and p = 0.833, respectively). There was no significant association between the frequency of pain and discomfort during or after intercourse or the degree of pain and discomfort and HIV status, nor for the ordinal scale values of signs of anal sensitivity and bleeding (direct notice of blood and blood on the toilet paper) in the MSM sample (Mann-Whitney U test; p = 0.247, p = 0.484, p = 0.621, p = 0.151, p = 0.951 and p = 0.731, respectively).

Discussion
Although HIV prevalence is decreasing [1], the drivers of ongoing HIV infections are yet to be addressed in more detail. The causal role of co-factors (STIs, intravaginal practices, intimate partner violence) seem unclear and biological transmission risk for infection remains essential among MSM [3, 6–8]. In this study, dyspareunia and signs of epithelial trauma were highly prevalent in FSWs and MSM, indicating considerable limitations to sexual health and personal well-being. Exposure to blood during sexual encounters may increase HIV transmission risk as evidenced by previous research [20, 27]. Vaginal coital bleeding in this study was more prevalent than previously described [17–19], and direct signs of anal bleeding were rife among MSM, justifying the need for further aetiological analysis for possible prevention measures.

Steady sexual relationships alongside sex work were common, which implies the importance of regular partner testing or considering pre-exposure prophylaxis given that HIV infections within heterosexual regular partnerships and unions classically outweigh the burden of HIV infections through every other mode of exposure in Kenya [29]. Artificial lubricant use was moderately popular with FSWs, indicating a possible benefit of lubricants for vaginal intercourse, confirmed by the sex workers’ favourable subjective assessment of lubricants as a remedy against dyspareunia. Lubricants, in combination with condoms, have been recommended for MSM [8] and have found high acceptance with Black American women [30]. Intravaginal substance insertion was rather common in the sex worker sample and linked to increased HIV acquisition risk, yet its large-scale impact as a driver of the HIV pandemic seems questionable according to meta-analyses [7]. Pain killers and sedatives were reported to be used in moderation but alcohol use was very common among the sex workers and this may be a possible risk factor for HIV transmission due to reduced self-care and precaution measures under the influence of alcohol. Alternatively, drinking and drug use may be a self-treatment for dyspareunia, and thus the association between HIV-seropositivity and sex drug or alcohol use may in turn be mediated via the infection risk from epithelial disruption linked to painful intercourse.

Contrary to the hypothesis that longer abstinence may subsequently lead to HIV acquisition, longer abstinence gaps in the previous month were positively associated with HIV-seronegativity among FSWs. This may be due to extremely reduced numbers of clients or much lower risk-taking behaviour among cautious HIV-negative participants. Some HIV-positive sex workers may, in turn, take fewer precautions and abstinence breaks, which, however, may not reflect their abstinence intervals at the time around their actual HIV infection as participants had contracted HIV earlier than the previous month. For the longest abstinence gaps in adult life from memory, there was no significant association with any HIV status, neither among FSWs nor MSM, so the curious association with abstinence remains unclear. Determining the role of abstinence intervals would require determining abstinence behaviour at the time of the actual HIV acquisition in the past, which was an impossible task within this study approach. Therefore, a lack of significant association between longest memorized abstinence gaps in adult life and HIV status may not necessarily contradict the hypothesis of shorter abstinence gaps or higher intercourse regularity as protective against HIV acquisition.
We found that later sexual debut may protect against HIV infection, which is somewhat intuitive. The apparent protection against HIV acquisition by having anonymous partners is not clear and needs further exploration. The apparent protection against HIV infection by foreplay may be explained by more relaxed tissues and better lubrication and hence less epithelial trauma, reducing the efficiency of HIV infection.

The hypothesis linking sexual dysfunction and epithelial trauma signs to HIV infection status could be confirmed since dyspareunia levels and frequency, as well as epithelial trauma signs and relationship dissatisfaction, were positively associated with positive HIV status. The temporal or possibly causal direction of this suggested link remains debatable given the cross-sectional study design, and further investigation is needed.

The subjective assessment of dyspareunia factors by FSWs implies modes of prevention against the sexual dysfunction. Steady partnerships seem to be beneficial as FSWs rather unanimously agreed that discomfort occurs less with a steady partner than a casual one. Whatever their protective mechanism, they are difficult to maintain for FSWs, people with a promiscuous sex life or unable to enter steady relationships, or in social contexts favouring concurrency. As for more experience with sex partners and having several partners at once, no clear recommendation can be drawn from the sex workers’ assessment for the prevention of dyspareunia. Longer duration of intercourse may worsen discomfort so that extremely prolonged sex may be recommended against. Higher regularity of intercourse, foreplay and lubricant use may, however, be recommended as protective as the women agreed that these factors may ease sexual dysfunction. The ideal maximum abstinence gaps between receptive sex appear unclear at the time of writing, and further investigation is needed. Interviewer and confirmation bias cannot be ruled out for the consensus of sex regularity as preventing painful intercourse, and blinded interviewing in further studies may be advisable to corroborate or refute the links to HIV risk and overall sexual health. Condoms seem not to interfere as most women judged their effect on dyspareunia to be irrelevant, so their role as an effective means to HIV prevention may be upheld. Finally, alcohol or sedative and analgesics use may increase HIV infection risk as seen in the inferential statistics, and there was no subjective agreement that drugs or alcohol would ease dyspareunia in any way.

The study revealed significant associations among FSWs for known HIV risk-taking behaviour such as early sexual debut, intravaginal substance insertion, alcohol and drug use as well as for the new link between sexual dysfunctions and HIV serostatus. The latter was self-reported, which helped establish a trusting relationship with participants but brought less reliability and objectivity for the HIV status variable. Among MSM, who also self-reported HIV status and were interviewed in the same fashion as sex workers, no similar significant results were found for anal dyspareunia. This difference may be due to more variation in the extent of vaginal sexual dysfunction in the sample because of higher variability of disorders of lubrication, arousal, of psychological confounding factors or physiological vaginal variation, co-infections or bacterial vaginosis. Gynaecological or rectal examinations and laboratory tests were not performed. The Nairobi population is culturally and ethnically diverse, and the role of genetics and different ethnic traits may be taken into consideration in further studies. Differences in vaginal anatomy were found between African-American and Caucasian women [31], and Frank Plummer had observed in
his early HIV immunity research that many highly exposed persistently seronegative FSWs were related to one another [32]. As for anal HIV transmission risk, matters of anatomy and physiology may be more uniform in nature so that differences in epithelial disruption may be more elusive and harder to differentiate in a sample because related complaints and signs may in turn be more uniform among MSM than among heterosexual women.

Conclusions

Recurrent exposure to blood during sexual intercourse and other minor epithelial disruption signs were highly prevalent in the HIV key population samples, as was recurrent painful intercourse, which may impact overall sexual health and bear a risk for HIV transmission. Significant associations between various aspects of sexual dysfunction and HIV infection status were found, which may be evidence that HIV transmission risk is linked to painful intercourse, to sexual life dissatisfaction, and to signs and symptoms of epithelial trauma. More foreplay, later sexual debut, the reduction of intravaginal practices, of alcohol consumption and of sedative and analgesic drug use may protect against HIV acquisition while the exact mechanisms, causal directions and cross-links of these factors require further exploration. Aetiological analysis on sexual dysfunction and minor epithelial trauma may help develop innovative prevention strategies against ongoing HIV transmissions. Unanimous subjective assessments on dyspareunia factors by female sex workers indicate that prevention may include the promotion of sexual intercourse regularity, foreplay, and artificial lubricant use after rechecking their respective protection against HIV acquisition. Based on our findings, longitudinal studies on HIV infection risk may benefit from considering these behavioural variables and the level of sexual dysfunction. Analysing epithelial trauma and sexual dysfunction as potential HIV infection risks may have to be more fine-grained for anal transmission than for vaginal transmission in order to find significant correlations.

Future studies on sexual dysfunction and HIV risk may advantageously be extended to key populations in different settings and to general populations with a high HIV prevalence, and next steps for research programs may include examining underlying biological factors. The study proposes novel opportunities for HIV prevention and sexual health improvement.

Abbreviations

MSM: men who have sex with men

STI: sexually transmitted infection

FSW: female sex worker

CTL: cytotoxic T lymphocyte

Declarations
Ethical Approval

Ethical approval was obtained from the Ethical Research Council of the Kenyatta National Hospital in June 2019 (KNH-ERC/A/216, P160/02/2019).

Consent to Participate

Written informed consent was obtained from all individual participants included in the study.

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors’ Contributions

LK and BF designed the study protocol. LK served as lead researcher on site in Nairobi, coordinated the project with the University of Nairobi and all other authors. LK and IM did the field coordination and data collection. BM and EK prepared the statistical analysis and data interpretation. BF conceived the research hypothesis and drafted the manuscript. WJ supervised the project, guided and revised the analysis and presentation of data and the preparation of the manuscript. All authors read and approved the final manuscript.

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