Effect of HSV-2 serostatus on acquisition of HIV by young men: results of a longitudinal study in Orange Farm, South Africa.

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Abstract

Background

The objectives of this study were to assess the role of HSV-2 status, first, on HIV acquisition by young men and secondly, on the protective effect of male circumcision on HIV acquisition.

Methods

We used data collected during a male circumcision trial conducted in Orange Farm (South Africa). We estimated adjusted incidence rate ratios (aIRR) using survival analysis and background characteristics, HSV-2 status, male circumcision status and sexual behavior as covariates.

Results

Compared to HSV-2 negative participants, HIV aIRR was 3.3 [95%CI, 1.5–7.4] P=.004 among those who were HSV-2 positive at enrollment and 7.0 [95%CI, 3.9–12.4] P<.001 among those who became HSV-2 positive during follow-up. The population fraction of HIV incident cases attributable to HSV-2 was 27.8% [95%CI, 17.7%–37.2%]. The intention-to-treat protective effect of male circumcision on HIV acquisition was the same among HSV-2 positive and HSV-2 negative men (0.38vs.0.37; P = .93).

Conclusions

This study shows that HSV-2 has a substantial impact on HIV acquisition among young South African men. It suggests that HSV-2 infection enhances HIV acquisition and is responsible for about a quarter of HIV incident cases. However, the protective effect of male circumcision on HIV acquisition appears independent of HSV-2 serostatus.

MESH Keywords Adolescent ; Antibodies, Viral ; blood ; Circumcision, Male ; HIV Infections ; epidemiology ; etiology ; prevention & control ; HIV-1 ; Herpes Genitalis ; complications ; epidemiology ; immunology ; Herpesvirus 2, Human ; immunology ; Humans ; Incidence ; Longitudinal Studies ; Male ; Risk Factors ; South Africa ; epidemiology ; Young Adult

Introduction

Genital herpes is a major public health problem worldwide [1]. Genital herpes is most frequently caused by the Herpes Simplex Virus type 2 (HSV-2). Clinical, biological and epidemiological studies support the hypothesis that HSV-2 increases the risk of HIV-1 acquisition 2- to 4-fold [2 –10] and increases the risk of HIV-1 transmission [2 , 11 –14]. A recent meta analysis found that genital ulcer disease associated with HSV-2 significantly increased the odds of HIV-1 detection in genital shedding (OR, 2.4 [95% confidence interval {CI}, 1.2 –4.9])[15].

HIV, in turn, increases the risk of HSV-2 transmission [2]. There is a direct relationship between CD4+ cell count and the rate of HSV-2 reactivation. Outbreaks of HSV- 2 are more severe, extensive, persistent, and invasive for those with advanced HIV disease [14 , 16]. Persistent HSV-2 infection was one of the original opportunistic infections that resulted in the identification of AIDS [17].

Three randomized controlled trials have demonstrated that male circumcision reduces the female-to-male sexual transmission of HIV by about 60% [18 –20]. A meta-analysis of observational data showed that male circumcision reduces the risk of HSV-2 infection with a borderline statistical significance (summary RR, 0.88 [95% CI, 0.77–1.01]) [21].

The main objectives of this study were to estimate a) the effect of HSV-2 status on HIV incidence among young males b) the population fraction of HIV infections attributable to HSV-2 infection and c) the effect of HSV-2 status on the protective effect of male circumcision on HIV acquisition by men. The secondary objective was to study the risk factors of HSV-2 incidence, especially the effect of male circumcision.

For these analyses, we used data collected during a male circumcision randomized controlled trial conducted in Orange Farm (South Africa), which demonstrated a reducing effect of male circumcision on the acquisition of HIV [18].

Methods

Collection of data

The technical details of the trial (ANRS-1265), description of the participants and HIV testing methods having been published elsewhere [18], only a summary will be presented in this article. Between February 2002 and July 2004, 3274 uncircumcised males, aged 18 to 24, were recruited, randomized into two groups and followed-up. Recruitment was conducted independently of HIV and HSV-2 status. Male circumcision was offered to the intervention group immediately after randomization and to control group participants after the end of the follow-up period. During each follow-up visits at 3 (M3), 12 (M12) and 21 (M21) months, circumcision status was ascertained by a nurse by genital examination and a blood sample was obtained. In addition, information about sexual behavior was collected, including number of sexual partners as a function of time and history of condom use with each sexual partner.

The dataset used in this study included 590 additional 21-month follow-up visits (20.0% of the total number of 21-month visits) which were not included when the analysis of the effect of male circumcision on HIV was published because corresponding biological data were not available at that time.

Laboratory methods

Plasma specimens were extracted from each blood sample immediately after collection, frozen at −20°C and kept frozen until processing. They were tested using an HSV type 2 specific IgG assay to detect HSV-2 antibodies with an index cutoff value of 1.1 (Kalon HSV-2 gG2 assay; Kalon Biologicals Ltd., Aldershot, UK), according to the manufacturer's recommendations.

Data analysis

HIV status, treated as censored data with time being continuous, was established at each follow-up visit for periods of non-uniform duration. These data were modeled using a piecewise exponential proportional hazards model in which the baseline hazard was considered constant between consecutive follow-up visits. This theoretical model was established to take into account the precise duration between each visit as well as the time- dependent covariates. It was implemented by running a Poisson log-linear model on a dataset with each line corresponding to one of three periods of follow-up: from randomization (M1) to M3, from M3 to M12 and from M12 to M21. During these periods participants remained HIV negative or became HIV positive [22 –24]. HIV incidence rates (IRs) and incidence rate ratios (IRRs) were thus estimated for the intervention and control groups (intention-to-treat analysis, ITT) and for circumcised and uncircumcised men (as-treated analysis, AT).

Adjusted IRRs were obtained by including in the analysis covariates that were collected for each period when they were time-dependent. The multivariate model included a) the period number, treated as a categorical variable, with the logarithm of the duration of exposure, in days, reported for each period as an offset, b) the participants' sociodemographic factors, c) behavioral time-dependent covariates and d) HSV-2 status coded in three categories (stayed HSV-2 negative, became HSV-2 positive during follow-up, was HSV-2 positive at enrollment).

The participants' sociodemographic factors were age, religion, ethnic group and alcohol consumption. The reported sexual behavior covariates were, for each period of follow-up, sexual risk behavior (defined as having at least one sexual contact unprotected by a condom), having a spousal partner and number of non-spousal sexual partners.

To assess the relative importance of HSV-2 seropositivity on HIV incidence among young men, the proportion of HIV incident cases attributable to HSV-2 seropositivity was computed using the formula given by Bruzzi and colleagues [25]. 95% confidence intervals (95 % CI) were obtained by bootstrapping.

The effect of HSV-2 status on the protective effect of male circumcision on HIV acquisition by men was assessed by testing the corresponding interaction term.

The piecewise exponential proportional hazards model was repeated to identify the risk factors of HSV-2 incidence.

In order to account for 1) the median time to seroconversion (window period) estimated at 120 days for the assay used [26] and 2) the healing period following male circumcision, the effect of male circumcision on HSV-2 was analyzed for the period M12 – M21. This period included all those tested for HSV-2 at least 240 days after male circumcision. The other factors were studied for the period M1–M12.

Furthermore, since HIV infection is reduced by male circumcision [18 –20] and associated with HSV-2 infection [27 , 28], the analyses of the association between HSV-2 and male circumcision were repeated excluding those who HIV seroconvert during follow-up in order to determine whether the effect of male circumcision on HSV-2 was affected by HIV acquisition during follow-up.

Lastly, the association between HSV-2 incidence and health-seeking behavior, defined as at least one visit to a clinic for a genital problem during the 12-month period prior to a visit to the centre, was studied separately, univariately and multivariately, using male circumcision status and the cofactors listed above.

The confidence intervals of the percentages were calculated using Bayesian estimation [29]. Statistical analyses were performed using the statistical package SPSS for Windows version 8 (SPSS, Chicago, Illinois, United States) and R (version 2.6.2) for analysis [30].

Complementary epidemiological data

To estimate HSV-2 prevalence by age and gender among the population of Orange Farm we used the data from a representative sample of 436 men and 476 women aged 15 to 49 from the community. These data were collected during a cross-sectional survey conducted in 2002 [31]. Blood samples were tested for HSV-2 serostatus using the same HSV-2 assay as the one used in the present study. For men and women, HSV-2 prevalences were 31.7% [95% CI, 27.4%–36.1%] and 67.7% [95% CI, 63.3%–71.7%], respectively. HSV-2 prevalence was 1.8% [95% CI, 0.28%–5.6%] among men aged 15 to 19 and 13.8% [95% CI, 7.9%–21.7%)] among men aged 20 to 24.

Ethics

The research protocol was reviewed and approved by the University of Witwatersrand Human Research Ethics Committee (Medical) on February 22, 2002 (protocol study no. M020104). The trial was also approved by the Scientific Commission of the French National Agency for AIDS Research (ANRS; protocol study no. 1265; 2002, decision No. 50) and authorization was obtained from the City of Johannesburg, Region 11, on February 25, 2002. This trial has been registered in http://www.clinicaltrials.gov under the number NCT00122525.

Results

Population characteristics

Among the 3274 participants enrolled in the trial, 5.9% were HSV-2 positive and 4.4% were HIV positive. 2974 participants were HSV-2 negative at enrollment and completed at least one follow-up visit. Among them, 67.2% were aged 20 and older, 48.1% were Sotho, 35.7% were Zulus, and 1.9% were married. Intervention and control groups did not differ in terms of baseline characteristics (HIV prevalence, 2.8% vs. 2.7%, proportion of those at sexual risk behavior, 46.0% vs. 46.0% and mean (median) number of lifetime sexual partners, 4.4 (4.0) vs. 4.4 (4.0), respectively).

HIV incidence and HIV incidence rate ratio

73 new HIV infections were diagnosed during follow-up. Table 1 indicates the effect of HSV-2 status during follow-up on HIV incidence: HIV incidence rate ratio was significantly higher among participants who were HSV-2 positive at recruitment and among participants who became HSV-2 positive during follow-up than among participants who stayed HSV-2 negative during follow-up. The HIV incidence rate ratio was about twice as high among those who became HSV-2 positive than among those who were HSV-2 positive at baseline, with a borderline level of significance, IRR, 2.2 [95% CI, 0.95–5.1]; P = .067 and aIRR, 2.1 [95% CI: 0.87–5.1]; P = .098.

Population attributable fraction

Of the 73 new HIV infections diagnosed during follow-up, 65.8% (48/73) [95% CI, 54.6%–75.9%] were among HSV-2 negative participants, 11% (8/73) [95% CI, 5.2%–19.4%] were among HSV-2 positive participants and 23.3% (17/73) [95% CI, 15.1%–34.2%] among participants who got infected with HSV-2 during follow-up.

Among circumcised and uncircumcised men, the percentage of HIV infections occurring among HSV-2 negative participants was similar, 64.9% (37/57) and 66.7% (10/15), respectively. The HSV-2 window period had a limited impact on this percentage: In effect, among the 33 HIV infections occurring during the M1–M12 period, 57.6% (19/33) [95% CI, 41%–73%] were among participants who remained HSV-2 negative during the M1–M21 period.

Using the proportion of participants among those who became HIV positive who were HSV-2 positive at recruitment or became HSV-2 positive during follow-up, 34.2% (25/73) [95% CI, 24.1%–45.4%]) and the corresponding aIRR, 5.3 [95%CI, 3.1–8.9], we estimated that 27.8% [95% CI, 17.7%–37.2%] of HIV incident cases in men were attributable to HSV-2 seropositivity or HSV-2 acquisition.

Effect of HSV-2 status on the protective effect of male circumcision on HIV acquisition

The protective effect of MC on HIV acquisition did not differ in terms of HSV-2 status (P = .93 for the interaction effect between randomization group and HSV-2 status). Among HSV-2 positive participants at baseline, the protective effect of male circumcision on HIV acquisition was 0.37 [95% CI, 0.09–1.55]; P = .17 (ITT) (0.20 [95% CI, 0.04–0.97]; P = .046 (AT)). This effect was 0.38 [95% CI, 0.22 –0.66] P < .001 (ITT) (0.24 [95% CI, 0.13–0.45]; P < .001 (AT)) among those who were HSV-2 negative. The protective effect of male circumcision on HIV among those who became HSV-2 positive during follow-up was 0.45 [95% CI, 0.16–1.27]; P = .13 (ITT) (0.28 [95% CI, 0.08–0.99]; P = .048 (AT)).

Risk factors of HSV-2 incidence

130 HSV-2 infections were diagnosed during follow-up: 102 (78.5%) [95% CI, 70.9%–84.9%] among HIV negative participants, 11 (8.5%) [95% CI, 4.5%–14.1%] among HIV positive participants and 17 (13.1%) [95% CI, 8.0%–19.6%] among participants who became HIV positive.

Table 2 presents the number of HSV-2 infections observed during follow-up and the univariate effect of male circumcision on HSV-2 incidence as a function of the randomization group and circumcision status. There was a borderline protective effect in the ITT analysis and the effect became stronger and significant in the AT analysis. When both analyses were repeated for the follow-up period M1–M21, the effect was, as expected, diluted, but still significant for the AT analysis: IRR, 0.87 [95% CI, 0.61–1.22]; P = .42 and IRR, 0.70 [95% CI, 0.49–0.99]; P = .044, respectively.

Table 3 presents the ITT and AT multivariate analyses of HSV-2 incidence. In both instances, the value of the protective effect of male circumcision and its level of statistical significance were close to the corresponding univariate values (Table 2). HSV-2 incidence was significantly associated with older age, sexual risk behavior and HIV status at enrollment. Table 4 illustrates the strong association of HIV status on HSV-2 acquisition during follow- up.

When those who HIV seroconverted during follow-up were excluded from the analyses, the univariate ITT and AT IRRs of HSV-2 incidence became 0.71 [95% CI, 0.41–1.25]; P = .24 and 0.63 [95% CI, 0.36–1.11]; P = .11. The corresponding aIRRs became 0.73 [95% CI, 0.39–1.38]; P = .34 and 0.50 [95% CI, 0.26–0.95]; P = .035. These values are close to the values reported in Tables 2 and 3 , indicating that the observed effect of male circumcision on HSV-2 acquisition cannot be due to the effect of male circumcision on HIV acquisition.

Attendance at a health clinic for a health problem related to the genitals

The percentage of participants who attended a clinic for a health problem related to the genitals in the past 12 months was 10.8% [95% CI, 9.7%–12.0%]. Participants who attended a clinic were more likely to have been recently infected with HSV-2 in the univariate analysis (IRR, 4.55 [95% CI, 2.94–7.02]; P < .001) and the multivariate analysis (aIRR, 3.31 [95% CI, 2.12–5.18]; P < .001).

Discussion

The primary aim of this study was to assess the role of HSV-2 status on HIV acquisition by men, using data collected during a male circumcision trial conducted in Orange Farm (South Africa).

We found a strong association between the incidences of these two viruses. Such association may be due to the fact that the viruses are both sexually transmitted and must share risk factors. It may also be due to the fact that one virus increases the risk of transmission of the other [2 , 11 –14].

This study was conducted in an area with high HIV and HSV-2 prevalences among adults[32 , 33]. As shown by the data of a cross-sectional survey, the relatively low HSV-2 prevalence among our sample is likely due to the fact that participants were young men. The population fraction of HIV incidence among young men due to HSV-2 in this study was around a quarter, value close to the lower bound of the estimates, ranging from 25% to 48%, obtained by modeling studies [34 , 35]. These results suggest an overall substantial effect of the HSV-2 status on the HIV epidemic. They further indicate that treating HSV-2 infection effectively could possibly prevent about a quarter of new HIV infections among males. However, recent research has shown that current acyclovir HSV-2 suppressive

therapy is not able to reduce HIV acquisition among HSV-2 positive women [36]. This can be explained by the fact that acyclovir does not cure HSV-2 infection.

Our results also suggested with a borderline significance level that HIV incidence may be twice as high among those who became HSV-2 positive than among those who were HSV- 2 positive at baseline. These results concur with those from a study, conducted among commercial sex workers in South Africa [9], which suggested that recent HSV-2 seroconversion appeared more important than established HSV-2 infection for the acquisition of HIV. Finally, we found that the protective effect of male circumcision on HIV acquisition was not altered by HSV-2 status or HSV-2 acquisition.

In terms of risk factor for HSV-2, our findings indicated that participants who were HIV positive at enrollment were more likely to acquire HSV-2. There was an independent and protective effect of male circumcision on HSV-2 acquisition by young males, which was significant only in the AT analysis. This effect was diluted and the statistical significance was lower in the ITT analysis, which can be attributed to the cross-over of 8.2% of participants in this study [18]. In the ITT analysis, the size of the protective effect of male circumcision on HSV-2 acquisition observed in this study was consistent with the results of a meta-analysis of observational studies which showed a relative risk of 0.88 [95% CI, 0.77–1.01] [21], and the preliminary results from the Rakai male circumcision trial [37]. However the AT analysis suggested a strong protective effect of MC against HSV-2 acquisition.

There are some limitations to this study: First, the window period of HSV-2 testing may impact the prevalence and incidence of HSV-2. Secondly, the crossover of participants was 8.2% and may explain the discrepancy between results from the ITT and AT analyses. Thirdly, the sample included young men among whom HIV and HSV-2 prevalence is relatively low compared to the general population, so our findings may not be generalized. Finally, the participants were taking part in a randomized controlled trial, so there may have been a selection bias.

This study provides additional evidence supporting the promotion of male circumcision in Africa: male circumcision has the potential to reduce the female-to-male transmission of HSV-2 and thus the spread of HSV-2, in addition to being a method proven to reduce the female-to-male transmission of HIV. The protective effect of male circumcision on HSV-2 is another argument in favor of rolling-out male circumcision in African countries were most males are uncircumcised [38].

Because of the complex interplay between HSV-2, HIV and male circumcision, further studies, in particular modeling studies, are needed to better understand their interactions; not only in the short term, as studied by randomized controlled trials, but also in the long term. Such studies should provide a comprehensive investigation of the epidemiological and geographical distribution of HIV and HSV-2 in Africa, taking male circumcision prevalence into account. Part of the effect of male circumcision on HIV may be attributed to its effects on HSV-2, such as the reduction of genital herpetic lesions which are associated with HSV-2 infection and may facilitate HIV transmission. However, this study contends that this effect is limited at population level.

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Footnotes:

Potential conflicts of interest

None reported.

Conference

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Contributions

JT and BA analyzed the data and wrote the first draft. DT organized the collection of the samples. AP analyzed the samples. All authors contributed to the writing.

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Table 1

HIV incident cases among men HIV negative at baseline and effect of HSV-2 on HIV incidence.

	HIV infection	HIV IRR	HIV aIRR
Stayed HSV-2 negative	48/2774 (1.7%)	1	
HSV-2 positive	8/122 (6.6%)	4.0 (1.9 – 8.4) P<0.001	3.3 (1.5 – 7.4) P=0.004
Became HSV-2 positive	17/119 (14.3%)	8.8 (5.0 – 15.0) P<0.001	7.0 (3.9 – 12.4) P<0.001

IRR= incidence rate ratio

aIRR= adjusted for the circumcision status, age, religion, ethnic group, alcohol consumption, sexual risk behavior, marital status and non-spousal partners (see notes in Table 3)

Table 2

HSV-2 seroincidence and univariate effect of male circumcision in the period M12-M21 of follow-up.

Type of analysis	HSV-2 cases	Follow-up (py)	HSV-2 IR (95%CI; per 100 py)	HSV-2 IRR (95%CI; P)
Intention-to-treat				
Control	35	1003	3.54 (2.54 – 4.93)	1
Intervention	23	995	2.33 (1.55 – 3.51)	0.66 (0.39 – 1.12) P=0.12
As-treated				
Uncircumcised	37	985	3.81 (2.76 – 5.26)	1
Circumcised	21	1012	2.09 (1.36 – 3.21)	0.55 (0.32 – 0.94) P=0.028

IR= incidence rate
py=person-year
IRR=incidence rate ratio

Table 3

Multivariate risk factors of HSV-2 seroincidence.

		Intention-to-treat IRR (95% CI)[1,2]	As-treated IRR (95% CI)[1,2]
Randomization group[3]	Control	1	NA
	Intervention	0.68 (0.38 – 1.22) P=0.20	
Circumcision status [3]	Uncircumcised	NA	1
	Circumcised		0.45 (0.24 – 0.82) P=0.0096
Age group	More than 21 years	2.34 (1.55–3.54) P<0.001	2.38 (1.58–3.60) P<0.001
	Less than or equal to 21 years	1	1
Religion	Catholic or Protestant	1.02 (0.57–1.83) P=0.96	1.02 (0.57–1.84) P=0.94
	Other	0.93 (0.63–1.37) P=0.72	0.91 (0.62–1.35) P=0.65
	African traditional	1	1
Ethnic group	Zulu	1.30 (0.86–1.95) P=0.21	1.28 (0.85–1.92) P=0.24
	Other	1.54 (0.95–2.50) P=0.082	1.59 (0.98–2.58) P=0.062
	Sotho	1	1
Drank alcohol in the previous month	Yes	1.19 (0.83–1.71) P=0.35	1.20 (0.83–1.72) P=0.34
	No	1	1
HIV status at enrollment	Positive	2.43 (1.25–4.72) P=0.009	2.48 (1.28–4.81) P=0.0074
	Negative	1	1
Sexual risk behavior[4,5]	Yes	1.85 (1.20–2.86) P=0.006	1.87 (1.21–2.89) P=0.005
	No	1	1
Married or living as married[5]	Yes	1.60 (0.85–3.00) P=0.14	1.63 (0.87–3.05) P=0.13
	No	1	1
Number of non-spousal partners[6]	>1	1.40 (0.91–2.16) P=0.12	1.42 (0.92–2.18) P=0.11
	0 – 1	1	1

IRR= incidence rate ratio

NA= Not applicable

1 Obtained using a piecewise exponential model, which was implemented with a Poisson log-linear model. Duration of exposure was the duration of each period for those staying HSV-2 negative and the duration of half the period for those becoming HIV positive.

2 Adjusted for all variables indicated in the first column

3 Using the M12–M21 period

4 Defined as having at least one sexual contact not protected by condom.
5 At some time in the past 3-month period before M3, and in the past 9-month period before M12 and M21.
6 In the past 3-month period before M3, and in the past 9-month period before M12 and M21.

Table 4

HSV-2 incident cases among men HSV-2 negative at baseline and effect of HIV on HSV-2 incidence.

	HSV-2 infection	HSV-2 IRR	HSV-2 aIRR
Stayed HIV negative	102/2828 (3.6%)	1	1
HIV positive	11/81 (13.6%)	4.1 (2.2 – 7.6) P<0.001	2.8 (1.5 – 5.5) P=0.002
Became HIV positive	17/65 (26.2%)	7.7 (4.6 – 12.8) P<0.001	5.9 (3.4 – 10.2) P<0.001

IRR= incidence rate ratio
aIRR= IRR adjusted for the circumcision status, age, religion, ethnic group, alcohol consumption, sexual risk behavior, marital status and non-spousal partners (see notes in Table 3)