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Changes in plasma viral load and penile viral shedding after circumcision among HIV-positive men in Kisumu, Kenya

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Abstract

Background—We conducted a prospective cohort study of HIV-positive men ages 18-35 years in Kisumu, Kenya to determine if medical circumcision of ART-naïve HIV-positive men leads to increased viral load and penile viral shedding.

Methods—From 108 HIV-positive men circumcised by forceps guided method and followed-up weekly for six weeks, 29 were evaluated for penile viral shedding. HIV-1 RNA was measured in plasma from 19 men and in penile lavage samples from 29 men. Samples were collected before circumcision and at weekly intervals for six weeks or until the circumcision wound was healed. CD4+ t-cell counts from 102 HIV-positive men were determined at baseline and at 2 weeks thereafter. Wounds with healthy scar, no scab or opening and no suture tracks were deemed healed.

Results—Among 65 ART-naïve men, mean CD4+t-cell count increased from 417 cells/mm³ at baseline to 456 cells/mm³ after 2 weeks ($p=0.04$), but did not change in the 37 men on ART ($p=0.81$). There was no change in HIV plasma viral load ($p=0.36$), but penile viral shedding rose significantly within one week after circumcision then declined to undetectable levels by 6 weeks (MANOVA; $p<0.001$). In 28 of 29 men (96.6%), there was no detectable viral shedding after certification of wound healing.

Conclusion—Medical circumcision among ART-naïve HIV-infected men results in a transitory rise in penile viral shedding before complete wound healing, which should pose no additional risk of HIV transmission if men adhere to six weeks post-circumcision sexual abstinence and use condoms consistently.

Keywords

HIV viral shedding; plasma viral load; male circumcision; HIV-infected men; Kenya

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Introduction

Voluntary medical male circumcision is a proven intervention for reducing HIV incidence in heterosexual men.¹⁻³ However, widespread circumcision of HIV-positive men may mitigate the public health benefits of male circumcision (MC). HIV-positive men may have increased viral shedding after circumcision, thereby exposing their sex partners to higher risk of HIV infection.⁴ In the ongoing scale-up of MC, many HIV-infected men are likely to be circumcised without knowledge of their HIV status or if they choose circumcision for non-HIV prevention benefits such as improved hygiene, reduced risk of phimosis, paraphimosis, genital ulcer disease, HSV-2 and HPV.⁵⁻⁷ While the HIV testing rate among men seeking MC in Kenya is approximately 92%⁸, not all programs may be as successful; HIV-positive men will inevitably be circumcised with unknown HIV status. Additionally, according to WHO guidelines, clinically eligible men who test positive for HIV should not be denied circumcision.⁹ During Kenya's first rapid results initiative for jumpstarting MC program in 2009, about 79% of the men who tested HIV-positive and knew their test results opted for circumcision.¹⁰

There is limited data on the effect of MC on plasma viral load or penile viral shedding, both of which may influence the transmissibility of the virus from HIV-infected men to their sex partners.¹¹⁻¹⁴ In addition to breaching dermal integrity, MC causes inflammation, which can lead to increased penile viral shedding and increase the risk of HIV transmission.^{11, 15-17} Wawer et al. reported a significant increase in plasma viral load ($+0.20 \log_{10}$ copies/mL, $p=0.002$) within 4 weeks post-circumcision among ART-naïve men.⁴ It is possible that the post-circumcision increase in plasma viral load could lead to increased viral shedding and higher infectivity¹⁸⁻²⁰, but no study has reported the effect of circumcision on penile HIV viral shedding. This prospective cohort study was designed to assess changes in CD4+t-cell counts, HIV viral load and penile viral shedding after medical circumcision of ART-naïve HIV-positive men in Kisumu, Kenya.

Methods

Participants

This study was nested within a larger study which enrolled 215 HIV-negative and 108 HIV-positive men to assess wound healing and resumption of sex following MC of adult males by the forceps guided method.^{21, 22} HIV-positive participants were recruited among men seeking MC at the UNIM Research and Training Center of the Nyanza Reproductive Health Society (NRHS) and through referrals from local HIV testing centers and clinics that serve HIV-positive men. Plasma and penile lavage samples for viral load determination were collected from HIV-positive participants on the day of circumcision, at subsequent weekly follow up for seven weeks, and finally at week 12. Since plasma viral load did not change from baseline to week 6 post-circumcision in the first 19 men, additional assays were not run. Viral load assays in penile lavage were done for the same 19 men and, due to budgetary constraints, only for the next 10 enrolled ARV-naïve men. These sample sizes can detect $0.48 \log_{10}$ copies/mL change in plasma viral load and $0.38 \log_{10}$ copies/mL change in penile viral shedding at any time point at $\alpha = 0.05$ and power = 0.8. All participants were also screened for HSV-2 using Kalon HSV-2 IgG ELISA (Kalon Biological Limited, Andershot, United Kingdom). Participants provided written informed consent for each study procedure. The study was approved by the Institutional Review Board of the University of Illinois at Chicago and the Kenyatta National Hospital Ethics and Research Committee.

Procedures

Post-operative wounds were assessed by a clinician at each follow-up and certified healed based on healthy scar formation with no scab or opening along the incision line and no suture tracks.^{21, 22} At each follow-up, participants were asked by a study clinician in a face-to-face interview if they had engaged in sex since surgery. Men who answered yes were prompted to provide the date of first postsurgical sexual intercourse and to provide details regarding condom use, type of partner and HIV-related risk behaviors. Counseling on behavioral risk reduction including post-circumcision abstinence was reinforced at each follow-up. CD4+t-cell counts were performed on blood samples collected from 108 men just before circumcision and at 2 weeks post-surgery, using BD FACSCount™ (Dickinson and Company, Franklin Lakes, New Jersey). Venous blood and penile lavage samples for HIV-1 RNA assay were collected just before circumcision (baseline), and at weekly intervals through six weeks of follow-up or until the circumcision wound was fully healed. The blood samples were collected in EDTA bottles and centrifuged at 1200 rpm for 10 minutes. To collect penile lavage specimens, 5mls of phosphate buffered saline (PBS; pH7.2) was measured into a 50ml sterile cup and 2.5mls drawn in a fine tipped transfer pipette and used to irrigate the penile coronal area. This irrigation was repeated three times and the wash fluid drained back into the same 50ml cup each time.²³⁻²⁵ Plasma and penile lavage samples were aliquoted into 1ml sterile cryotubes then stored at -80° C until assay. At assay, after thawing, samples were vortexed and 1ml was ultra-centrifuged for 1 hour at 16,400 rpm at 4° C to pellet the virus. Viral pellets were re-suspended using 1,100µL of the 1× PBS and tested using Cobas Ampliprep/Cobas Taqman (CAP/CTM) version 2.0 with lower threshold for detection of 20 copies/mL. HIV-1 viral RNA was quantified using HIV-1 QS which compensates for effects of inhibition and controls the preparation and amplification processes. Samples less than 1,000ml were diluted 1 in 2 with 1× PBS and ultra-centrifuged to pellet the virus. Results were multiplied by the dilution factor. Viral load assays were done at CDC HIV-Research laboratory in Kisumu which is ISO 15189 accredited and whose viral load platform is certified by the Virology Quality Assurance Program (VQA) and College of American Pathologist (CAP).

Analysis

Statistical tests were done using STATA version 9.0 (StatCorp, College Station, Texas 77845 USA. <http://www.stata.com>) and SAS version 9.2 (SAS Institute, Cary, NC, USA). Change in CD4+t-cell count from baseline to two weeks post-circumcision was analyzed using mixed-effects regression to account for missing data. There being no missing values, Multivariate Analysis of Variance (MANOVA) was performed to assess change in HIV viral load in plasma and penile lavage following circumcision through six-weeks of follow-up. A correlation analysis was done to explore the relationship between plasma HIV viral load and penile viral shedding. Chi-square (χ^2) was used to assess the association between baseline CD4-t cell count (categorized as low or high using a cut-off of 350 cells/mm³) and ART and viral shedding, respectively.

Results

Baseline characteristics of the 108 study participants are summarized in Table 1. Median age was 28yrs (IQR: 25-32), 58.3% of the men were married, 56% reported having two or more sex partners in the past one year and 31.5% reported consistent alcohol intake at least once per week. Of the 46 (43%) men with CD4+ t-cell counts less than or equal to 350 cells/mm³ at baseline, only 16 (34.8%) were already on ART. Overall, 41 of 108 (38%) men were already on ART and 81 of 108 (75%) men tested positive for HSV-2.

CD4 T-cell count

CD4+ t-cell count results were available for 107 men at baseline and 103 men at week 2 post-surgery due to one invalid test result at baseline and five missing samples at week 2. For the 37 men already on ART, mean CD4+ t-cell count remained unchanged from baseline (393 cells/mm³) to 2 weeks post-circumcision (396 cells/mm³); $p=0.81$. Among ART-naïve men, CD4+ t-cell count increased from 417 cells/mm³ at baseline to 456 cells/mm³ at week 2 post-surgery ($p=0.04$). There was no correlation between CD4+ t-cell count and plasma viral load either at baseline ($r=0.02$; $p=0.93$) or at 2 weeks ($r=0.049$; $p=0.84$) for the 19 men whose blood samples were tested for both CD4+ t-cell and viral load.

HIV viral load in plasma

Figure 1 shows plasma viral load results for 19 ART-naïve men before circumcision and at weekly intervals thereafter for six weeks. There was a small, insignificant decrease in plasma viral load from baseline (4.86 log₁₀ copies/mL) to week 4 post-circumcision (4.65 log₁₀ copies/mL); $p=0.178$, and multivariate ANOVA showed no change in plasma viral load from baseline to 6 weeks after circumcision; $p=0.10$ (Table 2). One man stood out as an outlier with a progressive decline in plasma viral load throughout the period of follow-up. His CD4+ t-cell count rose from 300 cells/mm³ at baseline to 409 cells/mm³ after 2 weeks.

Penile HIV viral shedding

Penile viral shedding results by weekly intervals from the day of circumcision to 6 weeks post-surgery are presented for 29 ART-naïve men (Figure 2). Five out of the 29 men (17.2%) had detectable viral shedding from the penis before circumcision and 80% of these (4/5) had CD4+ t-cell counts below 350 cells/mm³ but were not yet on ART. Three of the 29 men who had no detectable viral shedding at baseline had no measurable virus at any time post-circumcision. The remaining 26 men had an upsurge in penile viral shedding which peaked at 1 week post-circumcision then declined to undetectable levels within 6 weeks in all but one individual, whose viral shedding became undetectable at week 7 (not shown in Figure 2). MANOVA from baseline to six weeks showed highly significant changes in viral shedding following circumcision (Table 2).

Baseline CD4 cell count (<350 vs ≥350 cells/mm³) was not associated with penile viral shedding (none vs any) (Fishers exact, $p=0.33$) nor was viral shedding associated with HSV-2 serostatus (Chi-square, $p=0.27$). Plasma viral load and penile viral shedding were correlated at week 1 ($r=.60$; $p=0.007$) and at week 2 ($r=.58$; $p=0.009$), but not thereafter.

The temporal sequence of clinical certification of wound healing, self-reported resumption of sex, and change in penile HIV viral shedding is shown in Figure 3. Viral shedding peaked at weeks 1 and 2 before any of the men resumed sex. Weeks 3-5 were characterized by a steep decline in viral shedding and increases in the proportion of men certified as healed or resuming sex. By week 6, 45% of men had resumed sex and 93% of men were certified as fully healed. Most importantly, in 96.6% (28/29) of the men, no viral shedding was detected after certification of wound healing. Only one man (3.4%) still had penile viral shedding from an unhealed wound when he first resumed sex; and he reported using a condom. Neither the mean viral load nor the proportion of men shedding virus differed between those who resumed sex early compared to those who delayed sex to six weeks.

Discussion

There is concern that widespread male circumcision programs for HIV prevention could increase the risk of HIV transmission from seropositive men to their sexual partners if the surgical procedure and subsequent wound healing processes result in elevated plasma viral

loads and increases in penile viral shedding.^{4,26} We found no change in CD4+ t-cell count from baseline to two weeks post-circumcision in seropositive men who were on ART and a slight increase among ART-naïve men from 417 to 456 cells/mm³. We found no increase in plasma viral load at any point during six weeks after circumcision, a finding that differs from a previous study by Wawer et al. in Rakai, Uganda, where plasma viral loads among ART-naïve men were elevated above baseline four weeks after circumcision by a mean of 0.20 log₁₀ copies/mL.⁴ While our sample size of 19 men was small, limiting power to detect significant changes, the viral loads of men in our study did not rise, but rather declined, albeit insignificantly, after circumcision from a mean of 4.86 log₁₀ copies/mL at baseline to 4.65 log₁₀ copies/mL at week 4 post-surgery. It is difficult to determine the reasons for the differences between our results and those from Rakai. The Rakai sample was restricted to men who had CD4+ t-cell counts >350 cells/mm³ and to those with detectable virus at baseline. Their baseline levels of plasma virus (mean 4.30 log₁₀ copies/mL) were somewhat less than those of the men in our study. If we restrict our analysis to the 10 men whose CD4+ t-cell counts were >350 cells/mm³ and virus at baseline was detectable, we still observe a mean viral load of 4.68 log₁₀ copies/mL at baseline with a slight reduction to 4.62 log₁₀ copies/mL at week 4 post-surgery.

Regarding penile viral shedding, five of 29 (17%) men had detectable HIV viral shedding at baseline. Viral shedding before circumcision could have been associated with scratches, abrasions or micro-lesions of the penile skin, which have been found to be frequent in this population, but these were not detected in this study.^{27, 28}

Despite detecting no change in plasma viral load after circumcision, we found that penile viral shedding increased dramatically to a peak of 2.41 log₁₀ copies/mL one week after circumcision and then declined steadily such that only one of 29 men had any detectable penile shedding at Week 6, and he had returned to undetectable levels by week 7. Although penile viral shedding was correlated with plasma viral load during weeks 1 and 2, the correlation did not persist thereafter, indicating that plasma viral load is unlikely to be a useful predictor of penile viral shedding during the crucial period when some men may resume sex with an unhealed wound. These findings are consistent with the many studies showing HIV-1 compartmentalization between blood and other tissues and they demonstrate that assessment of penile viral shedding is crucial for determining the risk of HIV transmission during the post-circumcision period.^{29,30} Given that in 96.6% of the men, no viral shedding was detected after full wound healing, it is reasonable to conclude that the post-circumcision upsurge in viral shedding was due primarily to local breach in epithelial integrity. We found no difference in viral shedding between men who resumed sex early and those who did not, suggesting that sexual intercourse does not result in a breach in the functional integrity of a healed wound to cause an upsurge in viral shedding.

Our finding that penile viral shedding returns essentially to baseline levels by week 6 post-surgery should be considered as welcome in view of the WHO guidelines recommending 42 days of sexual abstinence after medical circumcision.⁹ We have previously shown that 94% of men are fully healed by Week 6 post-surgery, and that, although approximately 38% of men resume sex before 42 days post-circumcision, only 7% had unprotected sex before they were certified as fully healed.^{21,22} Wawer et al. (2009) noted that the risk of HIV transmission due to sex before the circumcision wound is healed is brief and likely to be small in proportion to the number of new infections to be averted in the population over decades through the protective effect of male circumcision.⁴ Our results not only support this view, but suggest that the risks of HIV transmission to the partners of HIV-positive men are considerably less than previously considered.^{4, 31} Nevertheless, we have shown that 24% of ART-naïve HIV-positive men continue shedding virus above baseline up to four weeks post-surgery; thus developing effective counseling and communications strategies for

both men and their female partners to avoid sex before 42 days after circumcision and to optimize condom use remains an essential component of a comprehensive MC program for HIV prevention.^{9, 21}

Our study has a number of limitations. The men recruited for the study, while residents of Kisumu who were seeking MC, may not be representative of the full range of HIV-positive men in the community who could be served by the Kenyan MC program. All the study men accepted HIV testing; in the Kenya MC program 92% of men agree to be tested before circumcision. Because men in this study were counseled weekly, the participants may have been more motivated to properly care for their wound or to refrain from sex than those circumcised as part of the national MC program. Consequently, the timing and rate of unprotected sex reported here may be different from routine MC program settings, although the proportion of men we found resuming sex before 42 days post-circumcision is very similar to findings from two previous studies conducted among clients attending the Kenya MC program.^{32, 33} Our sample size, especially for plasma viral load, is small, limiting our power to detect significant changes over time. However, the changes that we observed were very small and, if anything, were downward. The data are sufficient to conclude that plasma viral load did not increase from before to after circumcision in these men in Kisumu. A further limitation is that we do not know if circumcision surgery causes increases in HIV in the ejaculate. It is not feasible to collect semen samples from study participants by asking them to masturbate with an open wound. Recently, post-prostatic massage was shown to be a feasible and valid method for assessing male genitourinary tract HIV shedding, but this was not attempted in this study.³⁴

In conclusion, we observed that CD4+ t-cell counts and plasma viral loads of ART-naïve HIV-positive men in Kisumu did not change after circumcision. There was, however, a surge in penile viral shedding within one week after surgery, but levels of viral shedding declined thereafter and became undetectable within six weeks. While our results indicate that the increased risk of HIV transmission by HIV-positive men due to surgical circumcision is small and transient during the six week post-circumcision window, WHO guidance for MC programs to emphasize post-circumcision sexual abstinence for six weeks and to promote consistent condom use should be reinforced.

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Figure 1. Weekly plasma viral load from baseline to week 6 after MC (n=19)

Figure 2.

Penile viral shedding detected in lavage at weekly intervals from baseline to 6 weeks post-circumcision (n=29). The error bars represent the 95% confidence intervals for the mean viral load in penile lavage. * Number of participants with no detectable viral shedding.

Figure 3. Proportion of HIV-positive men shedding detectable virus from penis, assessed as healed and reporting resumption of sex by weekly intervals (n=29).

Table 1

Baseline characteristics of 108 HIV-positive men whose CD4+ t-cell counts were determined at baseline and at two weeks post-circumcision and of the sub-sample of 29 men whose penile viral shedding was assessed weekly for six weeks.

	HIV ⁺ (n=108)	Men assessed for viral shedding (n=29)	$\chi^2(p\text{-value})^+$
Age (years)			
18-24	22 (20.4%)	6 (20.7%)	0.02 (0.99)
25-29	44 (40.7%)	12 (41.4%)	
30 ⁺	42 (38.9%)	11 (37.9%)	
Marital status			
Single	45 (41.7%)	14 (48.3%)	0.39 (0.53)
Married	63 (58.3%)	15 (51.7%)	
CD4 Count (cells/ μ L) [*]			
350	46(43%)	14 (48.3%)	0.31 (0.58)
>350	61(57%)	15 (51.7%)	
ART at enrollment			
No	67(62.0%)	29(100.0%)	Not applicable
Yes	41(38.0%)	0	
HSV-2 serology			
Positive	81 (75.0%)	23(79.3%)	0.14 (0.71)
Negative	27 (25.0%)	6 (20.7%)	
Sexual partners in past year [¶]			
None or one	47 (44.0%)	14 (48.3%)	1.35 (0.51)
Two	27 (25.2%)	5 (17.2%)	
Three or more	33 (30.8%)	10 (34.5%)	
Alcohol use (days per week)			
None	63 (58.3%)	15 (51.7%)	0.52 (0.92)
Less than one	11 (10.2%)	4 (13.8%)	
One to two	20 (18.5%)	6 (20.7%)	
Three or more	14 (13.0%)	4 (13.8%)	

⁺ χ^2 values apply to the difference between the 29 study men and the 79 men not included in assessment for viral shedding.

^{*} One man had invalid CD4 cell count results at baseline hence n=107

[¶] Missing data for sexual partners for one participant hence n=107

^{**} Men not on antiretroviral treatment at baseline were selected for viral shedding analysis

Table 2
Change in CD4+ t-cell count, plasma viral load and penile viral shedding following circumcision: Results of mixed effect regression for CD4 cell count and MANOVA for plasma viral load and penile viral shedding

Change in CD4 cell count from baseline to 2 weeks for men on ART			
Observations	Mean CD4 cell count	[95% CI]	
Week 0	393	320-466	p=0.81
Week 2	396	323-470	
Change in CD4 cell count from baseline to 2 weeks among ART naïve men			
Observations	Mean CD4 count	[95% CI]	
Week 0	417	367-467	
Week 2	456	399-513	p=0.04
Change in plasma viral load following circumcision			
Week	Observations	Mean viral load	[95% CI]
0	19	4.86	4.57-5.16
1	19	4.66	4.38-4.97
2	19	4.59	4.19-4.99
3	19	4.64	4.36-5.01
4	19	4.65	4.25-5.04
5	19	4.57	4.14-5.00
6	19	4.53	4.08-4.99
			p=0.36
Change in penile viral shedding following circumcision			
Week	Observations	Mean viral load	[95% CI]
0	29	0.32	0.04-0.60
1	29	2.41	1.82-3.02
2	29	2.08	1.52-2.64
3	29	1.18	0.56-1.79
4	29	0.61	0.16-1.07
5	29	0.30	-0.07-0.67
6	29	0.08	-0.08-0.24
			p<0.01