

Nonoxynol-9 use, genital ulcers, and HIV infection in a cohort of sex workers

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Abstract

Objectives—To measure the associations between use of nonoxynol-9 (N-9) and incidence of genital ulcers, and incident ulcers and HIV seroconversion.

Methods—In a study of barrier contraceptive use and HIV infection, 273 female sex workers used condoms and 100 mg N-9 suppositories, and recorded sexual activity on coital logs. Genital ulcers were diagnosed clinically at monthly clinic visits. HIV infection was diagnosed by ELISA and Western blot. We calculated ulcer incidence rates by level of N-9 use. A nested matched case-control analysis assessed the effect of ulcers on HIV acquisition.

Results—More frequent N-9 use was not associated with genital ulcers and may have been protective against the lesions. Ulceration was not a strong risk factor for HIV acquisition in this study (odds ratio 1.1; 95% confidence interval 0.3-3.5).

Conclusions—Frequent use of N-9 can cause genital irritation and ulceration. Ulcers, in turn, may be risk factors for HIV acquisition. This study, however, did not find an association between N-9 use and ulcers, nor between ulcers and HIV. There is probably a threshold of N-9 use frequency or dose below which the risk of ulceration is minimal. Ulcers due to infectious causes may have been prevented by N-9 use in this cohort.

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Introduction

The role of nonoxynol-9 (N-9) in increasing the risk of HIV acquisition among women by causing disruption of the genital epithelium is still not clear and remains one of the important unanswered questions in HIV prevention research. In July 1990 a study among sex workers in Nairobi was prematurely terminated by a Data and Safety Monitoring Committee because the elevated HIV seroconversion rate among users of vaginal contraceptive sponges containing 1000 mg of N-9 was attributed to the higher incidence of vulvar ulceration among the sponge users compared with women using lubricating glycerin vaginal suppositories.¹

In 1991, a pilot study in Thailand found

that six of the 14 women using 150 mg N-9 vaginal suppositories at high frequencies (four times per day) had evidence of disruption of the epithelium and/or bleeding.² A larger follow-up study in the Dominican Republic also found that women who used 150 mg N-9 suppositories at high frequencies had a rate of epithelial disruption five times that of placebo users.³ However, the rate of epithelial disruption among lower frequency N-9 users was much lower. In fact, the rate among women using N-9 suppositories every other day was similar to the rate among women using a placebo. Unlike the Nairobi study,¹ neither the Dominican Republic nor Thailand studies found an increase in vulvar ulceration among frequent N-9 users.

Because the studies have not used the same vehicle for N-9 delivery, the same concentrations of N-9 in each dose, the same additional ingredients, the same clinical outcomes, or similar populations or frequencies of use, comparison of results is difficult. Thus important questions about whether there is a threshold level of use or dose above which the level of effect diminishes or changes direction remain unanswered.

In this analysis we assess whether the use of N-9 suppositories increased the risk of genital ulcers in a cohort of female sex workers in Cameroon and whether the development of genital ulcers increased the risk of HIV infection in the same cohort. As reported previously, the use of N-9 suppositories in this cohort was associated with a reduced risk of HIV infection.^{4,5}

Methods

A total of 303 sex workers were initially enrolled in the cohort study. Because the primary research question for the original study was the association between N-9 use and HIV, 30 women were excluded from the cohort because their ELISA tests (Behring Enzygnost HIV-1+2) were positive and confirmed by Western blot (Diagnostic Biotechnology; DuPont, Boston, Massachusetts, USA) either at admission or during the first three months. The remaining 273 seronegative women were asked to use condoms and 100 mg N-9 suppositories at every sexual encounter and to record daily sexual activity and use of condoms and N-9 suppositories on coital logs that were reviewed at monthly clinic visits. A pelvic examination with speculum was conducted monthly. Presence or absence of vulvar, vaginal, cervical and

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perianal ulcers were recorded on a standardised examination form. Examination light without magnification or colposcope was used to detect ulcerations. HIV testing was done every three months.

To assess the effect of N-9 on cervical and vaginal ulceration, we estimated the incidence of cervical and vaginal ulceration among "frequent", "intermediate" and "infrequent" N-9 users. The classification of exposure to N-9 for each participant was determined based on the coital log data. Reported sex acts were divided into three categories: acts where an N-9 suppository was used, whether alone or with a condom; acts where only a condom was used; and acts where neither a condom nor N-9 suppository was used. The cumulative number of sex acts in each category for each participant was determined by adding together the number of acts reported until the month when one of the following occurred: evidence of ulceration, missing coital log data or missing ulcer data, other loss to follow-up, or end of the 12 month study. Women were then grouped into three categories: "frequent N-9 users" who used N-9 a mean of more than 15 times per month; "intermediate N-9 users" who used N-9 a mean of 11–15 times per month; and "infrequent N-9 users" who used N-9 a mean of ten or fewer times per month. The classification of N-9 use was based on the number of times N-9 suppositories were used (with or without condoms) rather than the percentage because the number of coital acts and concurrent use of condoms and N-9 suppositories may have differed between women, and we wanted to compare rates of ulceration based on the absolute frequency of epithelial exposure to N-9.

To assess the effect of ulceration on HIV infection, we performed a nested case-control analysis similar to the approach reported by Laga *et al*⁶ in assessing the risk of non-ulcerative STDs on HIV acquisition. Cases were defined as women with HIV-1 seroconversion who had at least 6 months of follow-up before seroconversion and who had complete data on ulcers, gonorrhoea, and use of condoms and N-9 suppositories during the first four of those six months (the relevant exposure period for HIV infection). Four controls were chosen for each case by selecting the next four women enrolled after each case in the study who: (1) enrolled within 30 days of the case; (2) had complete gonorrhoea, ulcer, condom and N-9 data for the four month exposure period; and (3) remained HIV-1 negative throughout the study. The exposure period was set at four months as in the Laga study based on the HIV-1 antibody testing of the women every 3 months and the assumption that the time between infection with the virus and appearance of antibodies in most individuals is 6–12 weeks. The prevalence of ulceration (cervical, vaginal, or vulvar) within this four month period among the cases was compared with the prevalence among the controls. A conditional (matched) logistic regression analysis was done using SAS software to esti-

mate the effect of genital ulcers on HIV acquisition, controlling for the number of acts unprotected by condoms or N-9 during the exposure period.

Results

Demographic characteristics of the women at admission have been described elsewhere.⁴ The mean age of the cohort was 27 years and the mean number of sexual partners per week was 3. About a third of the women had evidence of genital ulceration at admission (table 1); almost 90% of the ulcers were on the cervix. There was only one perianal ulcer evident at baseline and none during follow up. Six women had evidence of vulvar ulcers at baseline; two additional women developed evidence of vulvar ulcers during follow-up. Further analysis of incident perianal or vulvar ulcers was not conducted owing to the low number of women who had evidence of perianal or vulvar ulcers during the study.

Effect of N-9 on cervical and vaginal ulcers

First we assessed the effect of N-9 on cervical ulceration. Of the 273 women seronegative at admission, 80 (29%) with evidence of cervical ulcers at admission were excluded from the prospective cervical ulcer analysis. Two women did not report any sexual partners during the brief period they were in the study before dropping out; they are also excluded. Included in the analysis were 191 women with no evidence of cervical ulcers at admission.

Forty percent of the 191 women ($n = 77$) were classified as frequent N-9 users; 44% ($n = 84$) as intermediate users; and 16% ($n = 30$) as infrequent users. Forty-two of the 191 women developed cervical ulcers. However, two of the women who had evidence of ulceration had prior incomplete data on ulceration or use of condoms and N-9. Because we censored these observations at the point of missing data, the incidence of cervical ulceration is based on 40 women with evidence of ulceration during 1,282 person-months of observation for an estimated incidence of cervical ulcers of 3.1 ulcers per 100 person-months of observation. (Women with no evidence of cervical ulceration contributed on average 9 person-months of data for the 12 month study.)

The incidence of cervical ulcers among frequent, intermediate, and infrequent N-9 users suggests that increasing use of N-9

Table 1 Prevalence of ulcers at baseline and cumulative incidence of ulcers during follow up

Ulcer site	Baseline		Follow-up	
	percent	N	percent	N
Vulvar	2.2	6/273	1.1	3/267
Vaginal	1.5	4/273	7.0	19/269
Cervical	29.3	80/273	21.8	42/193
Perianal	0.4	1/273	0.0	0/272
Any site	33.3	91/273	28.4	52/182

Note: measurements during follow-up are restricted to women without ulcers at baseline.

Table 2 Number and incidence of ulcers among frequent, intermediate and infrequent users of N-9 suppositories

	Frequent (>15 times per month)	Intermediate (11-15 times per month)	Infrequent (0-10 times per month)
Cervical ulcers			
Number	15	13	12
Incidence*	2.7	2.2	9.0
Vaginal ulcers			
Number	5	7	6
Incidence*	0.6	0.8	3.0

*Per 100 person-months.

suppositories was not associated with an increased incidence of cervical ulcers, and may have been protective against the lesions (table 2). Other explanations for infrequent N-9 users having a higher incidence of cervical ulceration were not readily apparent. Infrequent users had fewer sexual acts per month on average than other women; a similar number of acts per month protected by neither condoms or N-9; and a higher proportion of acts protected only by condoms. They used oral contraceptives (OCs) slightly more frequently than other women, suggesting possible misclassification of cervical ectopy associated with OC use as ulcers. However, when OC users were omitted from the analysis, infrequent N-9 users still had a higher incidence of cervical ulcers than moderate and frequent N-9 users (8.2 ulcers per 100 person-months vs 2.0 and 2.3 ulcers per 100 person-months respectively).

Next we looked at the effect of N-9 on vaginal ulceration. Of the 273 seronegative women, four women (1%) with evidence of a vaginal ulcer at admission and three who did not report any sexual partners before dropping out of the study were excluded from the prospective vaginal ulcer analysis.

Forty one percent of the remaining 266 women ($n = 110$) were classified as frequent N-9 users; 44% ($n = 117$) as intermediate users; and 15% ($n = 39$) as infrequent users. Nineteen of the 266 women developed vaginal ulcers. However, one of these women had prior incomplete data on use of condoms and N-9. Because we censored this observation at the point of missing data, the incidence of vaginal ulceration is based on 18 women who developed vaginal ulcers during the 1,995 person-months of observation for an estimated incidence of vaginal ulcers of 0.9 ulcers per 100 person-months of observation. (Women with no evidence of vaginal ulceration contributed on average 9 person-months of data for the 12 month study.)

The incidence of vaginal ulcers among frequent (0.6 ulcers/100 person-months), intermediate (0.8 ulcers/100 person-months), and infrequent (3.0 ulcers/100 person-months) N-9 users also suggests that use of N-9 suppositories was not associated with increased incidence of vaginal ulcers and that N-9 may have been protective against vaginal ulcers (table 2). As with cervical ulcers, other explanations for infrequent users having a higher incidence of ulceration were not readily apparent.

Table 3 Characteristics of cases and controls

	Cases	Controls
Characteristic		
Median age	26	26
<30 years old (%)	71	72
Less than primary education (%)	41	44
OC use at admission (%)	0	7
Median number of partners/month	15	16
Percentage of acts protected by condoms or N-9		
0-50%	0	0
51-75	29	19
76-90	53	49
91-100%	18	32
Genital ulcers during the exposure period (%)	29	28

Effect of ulceration on HIV acquisition

Two of the 19 women who became infected with HIV were excluded from analysis. One became infected during the first six months of the study; the other had incomplete condom and N-9 data for two of the four months of the exposure period. Four controls were identified for each of the remaining 17 cases. There were no apparent differences between cases and controls in terms of sociodemographic characteristics or number of sexual partners (table 3). Cases were more likely than controls to have unprotected coitus.

Five of the 17 cases (29%) had evidence of ulceration during the exposure period compared with 19 of 68 controls (28%). The estimated odds ratios for HIV infection for those with ulcers during the exposure period compared to those without ulcers was 1.1 (95% confidence interval 0.3, 3.5). When the number of acts unprotected by condoms or N-9 during the exposure period was added to the conditional logistic model, the odds ratio did not change.

Discussion

This exploratory analysis has two findings. First, use of 100 mg N-9 suppositories did not increase the risk of genital ulcers (cervical, vaginal, vulvar, or perianal) and was actually associated with a reduction in cervical and vaginal ulcers in this high risk population. This finding, although unexpected, is biologically plausible as N-9 suppositories may provide lubrication sufficient to prevent ulcers due to epithelial trauma during vigorous or "dry" sex (or both), either with or without a condom. N-9 suppositories may also be effective in preventing infection leading to ulcers (such as syphilis). Because we could not distinguish between ulcers due to N-9 and ulcers due to infection, however, we are reluctant to interpret these results as clear evidence of a protective effect. It is possible that among women not exposed to ulcerative diseases, that N-9 may have been associated with risk of ulceration.

These results do not conflict with previous studies associating use of N-9 with disruption of the epithelium or bleeding since the amount of N-9 per dose in this study was moderate (100 mg) and the frequency of use less than in other studies. The frequency of

N-9 use among "frequent" and "intermediate" users in this study is roughly comparable with the frequency of use among the women using N-9 every other day in the Dominican Republic study,³ in which rates of epithelial disruption and bleeding were similar to rates among the placebo group.

It is also noteworthy that there were fewer vulvar ulcers evident in this cohort compared with the Nairobi cohort.¹ The cervical and vaginal location of the ulcers in the current study is similar to the location of epithelial disruption and bleeding found in the N-9 toxicity studies,^{2,3} raising questions about whether the increased vulvar ulceration in Nairobi¹ may have been due to the higher dose of N-9 in the sponge, the sponge itself, or some other factor peculiar to the Nairobi population that was not present in Thailand, the Dominican Republic or Cameroon.

The second major finding is that genital ulceration was not a strong risk factor for HIV acquisition in this study, although it cannot be excluded as a moderate risk factor. Because the power of the study was low, owing primarily to the low number of HIV seroconversions in the cohort, a large confidence interval around the odds ratio was expected. However, such a low odds ratio (1.1) was not expected as genital ulcer disease has been identified as an important cofactor facilitating HIV acquisition in heterosexual and homosexual populations.^{7,8} There is no indication that ulcers were missed at a higher rate among women who later seroconverted or other evidence of misclassification.

There have been at least two mechanisms suggested by which ulcers may increase risk of HIV infection.⁸ They may provide a possible portal of entry for HIV. Second, in vitro evidence from immunological cell responses to organisms causing two ulcerative diseases, herpes simplex virus infection and syphilis, show that cells infected with these organisms are more susceptible to HIV infection than other cells.⁸ However, the possibility that use of N-9 suppositories reduces the risk of HIV acquisition due to ulcers should be considered. Ulcers caused by N-9 would not have the immunologic cell responses that increase susceptibility to HIV infection; and ulcers caused by syphilis might be less vulnerable to HIV passage in the presence of N-9, which is known to kill HIV.

There are several weaknesses of the current study. The lack of randomization to condom or N-9 use means that there may be unknown factors associated with risk of genital ulceration or HIV infection that differ substantially in their distribution between users of condoms and N-9. Secondly, because few women used N-9 infrequently or never and we wanted to have a sufficient number of women in the lowest use group, the "infrequent user" group includes women who used N-9 as often as 10 times per month. This would underestimate the protective effect of N-9. Thirdly, the specific cause of each ulcer was not identified, thus making it impossible to determine if an ulcer was due to an irritation effect of N-9 or to an infection. Fourthly, there was moderate loss to follow-up. Finally, the number of HIV seroconversions (17) used in the case-control analysis limited the ability of the study to estimate precise odds ratios.

Because ulcers have been associated with increased risk for HIV infection, the potential protective effect of N-9 at low and moderate levels warrants further study, particularly as women need access to methods of protection under their control from diseases identified as cofactors for HIV.

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