

VIRAL LOAD AND HETEROSEXUAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

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ABSTRACT

Background and Methods We examined the influence of viral load in relation to other risk factors for the heterosexual transmission of human immunodeficiency virus type 1 (HIV-1). In a community-based study of 15,127 persons in a rural district of Uganda, we identified 415 couples in which one partner was HIV-1–positive and one was initially HIV-1–negative and followed them prospectively for up to 30 months. The incidence of HIV-1 infection per 100 person-years among the initially seronegative partners was examined in relation to behavioral and biologic variables.

Results The male partner was HIV-1–positive in 228 couples, and the female partner was HIV-1–positive in 187 couples. Ninety of the 415 initially HIV-1–negative partners seroconverted (incidence, 11.8 per 100 person-years). The rate of male-to-female transmission was not significantly different from the rate of female-to-male transmission (12.0 per 100 person-years vs. 11.6 per 100 person-years). The incidence of seroconversion was highest among the partners who were 15 to 19 years of age (15.3 per 100 person-years). The incidence was 16.7 per 100 person-years among 137 uncircumcised male partners, whereas there were no seroconversions among the 50 circumcised male partners ($P < 0.001$). The mean serum HIV-1 RNA level was significantly higher among HIV-1–positive subjects whose partners seroconverted than among those whose partners did not seroconvert (90,254 copies per milliliter vs. 38,029 copies per milliliter, $P = 0.01$). There were no instances of transmission among the 51 subjects with serum HIV-1 RNA levels of less than 1500 copies per milliliter; there was a significant dose–response relation of increased transmission with increasing viral load. In multivariate analyses of log-transformed HIV-1 RNA levels, each log increment in the viral load was associated with a rate ratio of 2.45 for seroconversion (95 percent confidence interval, 1.85 to 3.26).

Conclusions The viral load is the chief predictor of the risk of heterosexual transmission of HIV-1, and transmission is rare among persons with levels of less than 1500 copies of HIV-1 RNA per milliliter. (N Engl J Med 2000;342:921-9.)

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IN sub-Saharan Africa, the predominant mode of transmission of human immunodeficiency virus type 1 (HIV-1) is through heterosexual contact, and the rate of transmission by this means is increasing throughout Asia and in many industrialized countries.^{1,2} A wide variety of behavioral and biologic risk factors are associated with the risk of transmission, including the frequency³⁻⁵ and types⁶ of sexual contact, the use or nonuse of condoms,^{5,7} immunologic status,⁸ and the presence or absence of the acquired immunodeficiency syndrome (AIDS),⁸ circumcision (in men),⁹⁻¹¹ and sexually transmitted diseases.^{6,12,13} Other potential factors include plasma HIV-1 RNA levels,¹⁴⁻¹⁷ the presence or absence of chemokine receptors,^{18,19} and the use or nonuse of antiretroviral therapy.²⁰ Improved understanding of the way in which these factors influence both the infectiousness of and the susceptibility to HIV-1 could facilitate efforts to prevent transmission of the virus.

To delineate the risk factors associated with heterosexual transmission of HIV-1 more clearly, we prospectively followed couples discordant for HIV-1 status in stable sexual relationships in a group of communities with a high prevalence of infection with HIV-1 (16.1 percent), mainly subtypes A and D. We were able to identify these couples retrospectively from a community-based trial of 15,127 persons residing in the rural district of Rakai, Uganda.²¹ We analyzed sociodemographic, behavioral, and biologic factors, with particular emphasis on the effects of serum viral load on the risk of heterosexual transmission of HIV-1.

METHODS

Study Population

The Sexually Transmitted Diseases Control for AIDS Prevention Study, a community-based randomized trial, was conducted in Rakai between November 1994 and October 1998. The design and results of the study have been reported previously.²¹ In brief, rural communities on secondary roads were aggregated into 10 clusters; 5 clusters were randomly assigned to receive intervention for sexually transmitted diseases, and 5 clusters were randomly assigned to a control group. Five community-based surveys were conducted at intervals of 10 months.

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Eligible persons were read a consent form that explained the study and its potential risks and benefits, and they were informed of their rights to decline all or part of the study activities without loss of access to clinical and educational services. The trial was approved by the AIDS Research Subcommittee of the Uganda National Council for Science and Technology, the human-subjects review boards of Columbia University and Johns Hopkins University, and the National Institutes of Health Office for Protection from Research Risk. Safety was assessed by an independent data safety and monitoring board.

Subjects in both groups received identical, intensive instruction on the prevention of HIV-1 infection and condom use and were offered free condoms and voluntary, confidential serologic testing for HIV-1 and counseling by trained project counselors. Since this was a community-based trial that enrolled all consenting adults, the identification of couples within the general population was done only retrospectively. Hence, our study differs from other investigations that selectively identified and followed HIV-1–discordant couples. During our study, individual and couples counseling was continually offered to all subjects, who were strongly encouraged to make use of this service, as recommended by the AIDS Control Programme of the Ugandan Ministry of Health.²² All subjects were also strongly encouraged to obtain the results of their tests for HIV-1 and to share the results with their partners, in accordance with the testing policy of the AIDS Control Programme.²² This policy explicitly states that “it is the right of the patient to decide who else to inform about the results” and thus precludes the “revealing [of the] results to sexual partners or spouses.”²² The policy also specifies that “medical personnel and anybody who has, during the course of their work, access to confidential information about the patient, does not divulge this information to third parties who are not directly involved in the care of the patient” and that, “because of the stigma and discrimination arising from HIV infection and AIDS, it is more important that everybody adheres strictly to this principle.”²²

Free condoms were made continuously available to the entire community. At each visit, health care was provided by Rakai Project mobile clinics, and subjects were advised to seek care in government clinics if they had symptoms that suggested the acquisition of sexually transmitted diseases between survey visits.

Subjects who were legally married or in consensual union, defined as a culturally accepted long-term sexual relationship, were asked to provide the name and address of the spouse or consensual partner. Such information was obtained for 75 percent of all eligible couples. During the first four surveys, we were able to identify 415 couples that were discordant for HIV-1 and that were together during the interval in which there was a risk of seroconversion. Rates of transmission and acquisition of HIV-1 were assessed in these couples.

Interviews and Tests

At base line and at each follow-up visit, subjects were interviewed separately and in private by same-sex interviewers to ascertain their sociodemographic characteristics; sexual behavior (the number of sexual partners in the past year and condom use); history of travel outside of the district; health history, including symptoms of genital ulcer disease, genital discharge, and dysuria that were present at the time of each interview and during the period between surveys; history of and treatment for sexually transmitted diseases; and the presence of AIDS-defining symptoms or conditions, according to the World Health Organization (WHO) criteria for a presumptive diagnosis.²³ The circumcision status of the male subjects was ascertained.

At base line and at each follow-up visit, all subjects were asked to provide a venous blood sample and a 10-ml first-catch urine sample, and the female subjects provided self-collected vaginal swabs; compliance was over 90 percent. Venous blood was tested for HIV-1 with two enzyme immunoassays (Vironostika HIV-1, Organon Teknika, Charlotte, N.C., and Cambridge Biotech, Worcester, Mass.), with confirmation of discordant results by Western

blotting (HIV-1 Western Blot, BioMerieux-Vitek, St. Louis). Syphilis was diagnosed with use of a commercial test (Toluidine Red Unheated Serum Test, New Horizons, Columbia, Md.), and positive samples were confirmed by treponemal-specific tests (TPHA Sera-Tek, Rujibero, Tokyo, Japan, or FTA-ABS IFA test system, Zeus Scientific, Raritan, N.J.). Urine samples were tested by a ligase chain reaction for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (LCx Probe System, Abbott Laboratories, Abbott Park, Ill.) in a subgroup of subjects. Among women, self-collected vaginal swabs were cultured for *Trichomonas vaginalis* (InPouch TV culture, BioMed Diagnostics, San Jose, Calif.) and examined morphologically for bacterial vaginosis with the use of Gram's staining.

The results of measurements of serum HIV-1 RNA were not available during the study. Archived serum samples from the couples were tested in batches approximately one year after the completion of the trial. Serum levels of HIV-1 RNA were quantified by a reverse-transcriptase–polymerase-chain-reaction assay (Amplicor HIV-1 Monitor 1.5 assay, Roche Molecular Systems, Branchburg, N.J.), as previously described.²⁴ This assay has been shown to quantitate all subtypes of HIV-1 reliably, including subtypes A and D, which are present in Uganda.²⁵ The limit of detection was 400 copies of HIV-1 RNA per milliliter, and samples with values below this limit were assigned a value of 399 per milliliter for the purpose of analysis.

Among couples in which the HIV-1–negative partner seroconverted, the HIV-1 RNA assay was performed on the serum sample obtained from the HIV-1–positive index partner at the study visit before the 10-month interval in which there was a risk of seroconversion (i.e., an average of 4 to 5 months before probable seroconversion). Couples in which there was no seroconversion were matched with couples with seroconversion according to the sex and age (within five years) of the HIV-1–positive and HIV-1–negative partners and the timing of the follow-up visit. For the couples that remained discordant, we selected from the HIV-1–positive partner the serum sample that was obtained closest in time to that of the matched seroconverting couple. Thus, the assays were frequency-matched according to sex, age, and the time at which samples were obtained in the case of both HIV-1–positive partners who transmitted the virus and those who did not.

Antiretroviral drugs are not available in rural Uganda. Consequently, the HIV-1 RNA levels were not influenced by the use of antiretroviral drugs.

Statistical Analysis

Descriptive analyses were conducted separately according to age and sex and the characteristics of the HIV-1–positive and HIV-1–negative partners. The presence or absence of sexually transmitted diseases was determined at the visit before and the one following the interval in which there was a risk of seroconversion, whereas information on behavior and symptoms of sexually transmitted disease during that interval were determined at the visit after the interval. At each visit, subjects were asked about current symptoms. The incidence of HIV-1 was estimated per 100 person-years in HIV-1–negative subjects and was based on the assumption that seroconversion occurred at the midpoint of the 10-month follow-up interval. The incidence was tabulated separately according to sociodemographic characteristics, behavior, and symptoms and diagnoses of sexually transmitted diseases in HIV-1–positive partners (i.e., to indicate the risk of transmission) and in HIV-1–negative partners (i.e., to indicate the risk of acquisition).

Tests of statistical significance included the 95 percent confidence intervals of the unadjusted rate ratios, two-sided P values based on chi-square tests or the chi-square test for trend, and Fisher's exact test.²⁶ Formal adjustments for multiple comparisons were not performed for associations based on a priori hypotheses (e.g., that transmission rates would increase with higher serum HIV-1 RNA loads, younger age, the absence of circumcision, and the presence of sexually transmitted diseases or their symptoms). In addition, we hypothesized that viral load would be higher among subjects who transmitted the disease to their partners than among

TABLE 1. RATES OF ACQUISITION AND TRANSMISSION OF HIV-1 ACCORDING TO AGE AND SEX.

GROUP	NO. OF SUBJECTS	NO. OF CASES/ PERSON-YR*	INCIDENCE/ 100 PERSON-YR	RATE RATIO (95% CI)†
All subjects	415	90/760	11.8	—
Sex of HIV-1–positive partner				
Male	228	50/415	12.0	1.0
Female	187	40/345	11.6	0.96 (0.63–1.46)
Age of HIV-1–negative partner				
15–19 yr	48	11/72	15.3	1.0
20–24 yr	82	18/136	13.2	0.87 (0.42–1.91)
25–29 yr	104	28/190	14.7	0.97 (0.50–2.03)
30–39 yr	107	18/215	8.4	0.55 (0.26–1.20)
40–59 yr	74	15/147‡	10.2	0.67 (0.31–1.50)
Age of HIV-1–positive partner				
15–19 yr	26	8/43	18.6	1.0
20–24 yr	99	24/172	14.0	0.76 (0.36–1.81)
25–29 yr	104	28/180	15.6	0.84 (0.40–1.98)
30–39 yr	130	20/250	8.0	0.43 (0.20–1.05)
40–59 yr	56	10/116§	8.6	0.47 (0.19–1.23)

*Person-years were estimated to two decimal places and were rounded to whole numbers.

†CI denotes confidence interval.

‡Chi-square for trend=0.65, P=0.45.

§Chi-square for trend=3.58, P=0.06.

those who did not. Mean and median viral loads were estimated on the basis of untransformed data and on data transformed to the base-10 logarithm. Viral loads were analyzed for the HIV-1–positive partners who transmitted the virus and for those who did not transmit the virus, and as well as according to age and sex. The t-test was used to compare mean viral loads.²⁶

Multivariate adjusted rate ratios for the risk of seroconversion were estimated with the use of Poisson regression analysis.²⁷ The viral load was the independent variable of interest and was assessed in separate models in which the actual viral load (copies per milliliter) was included as a categorical variable and the log-transformed viral load was included as a continuous variable. For the categorical variable, a serum HIV-1 RNA level of less than 3500 copies per milliliter was used as the reference category because there were no instances of seroconversion of HIV-1–negative subjects whose partners had HIV-1 RNA levels of less than 1500 copies per milliliter, and the rate ratios of HIV-1 seroconversion were estimated for viral loads of 3500 to 9999 copies per milliliter, 10,000 to 49,999 copies per milliliter, and 50,000 or more copies per milliliter. All models included terms for age (15 to 19, 20 to 29, 30 to 39, and 40 to 59 years) and for the sex of HIV-1–positive partners. The number of sexual partners in the past year (one vs. two or more), use or nonuse of condoms, circumcision or noncircumcision of the male partner, presence or absence of symptoms of sexually transmitted diseases (genital ulcer disease, genital discharge, and dysuria), and presence or absence of sexually transmitted diseases (syphilis, gonorrhea, and chlamydia in both sexes and trichomonas and bacterial vaginosis in women) were also assessed. Separate models were fitted for the characteristics of the HIV-1–positive partners and the HIV-1–negative partners.

RESULTS

Demographic Characteristics and Incidence of HIV-1

A total of 415 couples discordant for HIV-1 were enrolled between the first and the fourth survey and followed for a period of up to 30 months (median

follow-up, 22.5). The male partner was infected with HIV-1 at base line in 228 of these 415 couples (55 percent), and the female partner was infected in 187 (45 percent) (Table 1). Ninety (22 percent) of the HIV-1–negative partners seroconverted during the course of the study, for an overall incidence of 11.8 per 100 person-years. Fifty (56 percent) of the partners who seroconverted were female, and 40 (44 percent) were male. The rate of transmission from male partners to female partners was not significantly different from the rate of transmission from female partners to male partners (12.0 per 100 person-years vs. 11.6 per 100 person-years). The median age at enrollment was 30.3 years among HIV-1–negative partners and 29.4 years among HIV-1–positive partners (P>0.05). The highest incidence of seroconversion was among couples in the age group of 15 to 19 years (Table 1). The incidence declined with the age of both HIV-1–negative and HIV-1–positive partners, but these trends were not statistically significant (P>0.05).

Characteristics of HIV-1–Negative Partners Associated with the Acquisition of Infection

On bivariate analysis, there were no significant differences in the risk of infection among HIV-1–negative partners according to the level of formal education, history of travel outside the district within the previous year, the number of sexual partners within the past year (one vs. two or more), or condom use or nonuse. However, 364 of 407 HIV-1–negative partners for whom information was available (89 per-

TABLE 2. RATES OF ACQUISITION AND TRANSMISSION OF HIV-1, ACCORDING TO THE CHARACTERISTICS OF THE INITIALLY HIV-1-NEGATIVE PARTNERS AND THE HIV-1-POSITIVE PARTNERS.*

CHARACTERISTIC	HIV-1-NEGATIVE PARTNERS			HIV-1-POSITIVE PARTNERS		
	NO. OF SUBJECTS	NO. OF CASES/ PERSON-YR†	RATE OF ACQUISITION/ 100 PERSON-YR	NO. OF SUBJECTS	NO. OF CASES/ PERSON-YR†	RATE OF TRANSMISSION/ 100 PERSON-YR
Formal education						
No	43	9/85	10.6	29	6/50	12.0
Yes	372	81/676	12.0	386	84/711	11.8
Travel outside the district						
No	33	10/50	20.0	26	3/51	5.9
Yes	382	80/710	11.3	389	87/710	12.3
Circumcision						
No	137	40/239	16.7‡	195	46/349	13.2
Yes	50	0/106	0.0	29	3/58	5.2
Sexual partners in past yr						
1	324	74/591	12.5	291	60/544	11.0
≥2	91	16/169	9.5	124	30/217	13.8
Condom use in past yr						
No	364	77/671	11.5	356	74/660	11.2
Yes	43	12/74	16.2	55	15/62	24.2
Genital ulcer disease						
No	365	81/662	12.1	359	76/659	11.5
Yes	50	9/97	9.3	56	14/102	13.7
Genital discharge						
No	378	82/695	11.8	380	78/702	11.1
Yes	37	8/66	12.1	35	12/58	20.7§
Dysuria						
No	368	82/675	12.1	369	78/677	11.5
Yes	47	8/85	9.4	46	12/83	14.5†
AIDS-defining symptoms						
No	—	—	—	401	84/739	11.4
Yes	—	—	—	14	6/22	27.3§

*Only subjects for whom data were available are included.

†Person-years were estimated to two decimal places and were rounded to whole numbers.

‡P<0.001 for the comparison within the group.

§P<0.05 for the comparison within the group.

cent) never used condoms (Table 2). The rate of seroconversion among uncircumcised male subjects was 16.7 per 100 person-years, whereas no seroconversions occurred among circumcised male subjects ($P<0.001$). There were no significant differences in the rate of acquisition of HIV-1 infection according to either the presence or absence of symptoms of sexually transmitted diseases (Table 2) or the presence or absence of syphilis, gonorrhea, chlamydia, trichomonas, and bacterial vaginosis (data not shown).

Characteristics of HIV-1-Positive Partners Associated with the Transmission of Infection

Transmission rates were not significantly affected by the level of formal education, travel history, the number of sexual partners within the preceding year, or condom use or nonuse. Uncircumcised male subjects had a higher rate of transmission than circumcised male subjects (13.2 per 100 person-years vs. 5.2 per 100 person-years), but this difference was not statistically significant ($P=0.17$). On bivariate analysis, the laboratory diagnosis of sexually transmitted

diseases (data not shown) or genital ulcer disease did not significantly increase the rate of transmission. However, a history of genital discharge or dysuria in the HIV-1-positive partner was associated with a significantly increased transmission rate ($P<0.05$). The presence of AIDS-defining symptoms or signs was also associated with a significantly increased rate of transmission (27.3 per 100 person-years vs. 11.4 per 100 person-years, $P<0.05$). However, only 14 of 415 HIV-1-positive subjects (3 percent) met the WHO criteria for AIDS.

HIV-1 RNA Levels and the Risk of Transmission

Of the 415 seropositive partners, 364 (88 percent) had detectable serum levels of HIV-1 RNA. The mean serum level of HIV-1 RNA among the 228 HIV-1-positive men was 59,591 copies per milliliter (median, 15,649) and was significantly higher than the mean level of 36,875 copies per milliliter among the 187 HIV-1-positive women (median, 9655; $P=0.03$). When the log-transformed values were used, the mean (\pm SD) value was 4.11 ± 0.86 log copies of HIV-1

TABLE 3. MEAN AND MEDIAN VIRAL LOADS.

GROUP	ALL SUBJECTS			FEMALE SUBJECTS			MALE SUBJECTS			P VALUE*
	NO. OF SUBJECTS	MEAN \pm SD	MEDIAN	NO. OF SUBJECTS	MEAN \pm SD	MEDIAN	NO. OF SUBJECTS	MEAN \pm SD	MEDIAN	
	log copies of HIV-1 RNA			log copies of HIV-1 RNA			log copies of HIV-1 RNA			
All subjects	415	4.02 \pm 0.85	4.12	187	3.90 \pm 0.83	3.99	228	4.11 \pm 0.86	4.19	0.008
HIV-1-positive subjects with partners who seroconverted	90	4.48 \pm 0.64†	4.43	40	4.30 \pm 0.49†	4.27	50	4.62 \pm 0.69†	4.67	0.015
HIV-1-positive subjects with partners who did not seroconvert	325	3.89 \pm 0.86	4.00	147	3.79 \pm 0.87	3.83	178	3.97 \pm 0.85	4.12	0.06

*The P values are for the comparison with female subjects.

†P=0.001 for the comparison with HIV-1-positive subjects whose partners did not seroconvert (by Student's t-test).

RNA among the men and 3.90 \pm 0.83 log copies of HIV-1 RNA among the women (P=0.008) (Table 3). Among couples in which the initially HIV-1-negative partner seroconverted, the mean serum HIV-1 RNA level of the HIV-1-positive partner was significantly higher than that of the HIV-1-positive partner in couples in which the HIV-1-negative partner remained seronegative (mean, 90,254 copies per milliliter vs. 38,029 copies per milliliter; P=0.01). When these two subgroups were analyzed according to sex, the log-transformed values were significantly higher among male and female subjects whose partners seroconverted than among male and female subjects whose partners did not seroconvert (P=0.001) (Table 3).

There was a significant dose-response effect with respect to both male-to-female transmission and female-to-male transmission (P<0.001) (Fig. 1). The rate of transmission was zero among the 51 couples in which the HIV-1-positive partner had undetectable serum levels of HIV-1 RNA or less than 1500 copies per milliliter. Among HIV-1-positive partners with serum HIV-1 RNA levels of less than 3500 copies per milliliter, the rate of transmission was 2.2 per 100 person-years, and the rates progressively increased with increasing viral loads, to a maximum of 23.0 per 100 person-years at a level of 50,000 or more copies per milliliter. It is noteworthy that among the 90 instances of transmission, 5.6 percent occurred among couples in which the HIV-1-positive partner had serum HIV-1 RNA levels of 400 to 3499 copies per milliliter, 17.7 percent among couples in which the seropositive partner had levels of 3500 to 9999 copies per milliliter, 40.0 percent among couples in which the seropositive partner had levels of 10,000 to 49,999 copies per milliliter, and 36.7 percent among couples in which the seropositive partner had levels of 50,000 or more copies per milliliter. There was no significant difference between male-to-female and female-to-male transmission rates after the results were

adjusted for viral load (P=0.76), and there were no consistent differences between male-to-female or female-to-male transmission rates within strata of viral load (Fig. 1).

Results of Multivariate Logistic-Regression Analysis

We constructed several Poisson regression models, the results of which are summarized in Table 4. Viral load was the variable most strongly predictive of the risk of transmission. When viral load was measured as a categorical variable, with HIV-1-positive partners with serum HIV-1 RNA levels of less than 3500 copies per milliliter as the reference group, the rate ratio of the risk of transmission increased from 5.80 (95 percent confidence interval, 2.26 to 17.80) for HIV-1-positive subjects with HIV-1 RNA levels of 3500 to 9999 copies per milliliter to 11.87 (95 percent confidence interval, 5.02 to 34.88) for seropositive subjects with 50,000 or more copies per milliliter. When viral load was measured as a continuous variable, the rate ratio for the risk of transmission associated with each log increment in viral load was 2.45 (95 percent confidence interval, 1.85 to 3.26).

As compared with the risk of transmission among HIV-1-positive partners who were 15 to 19 years of age, the risk of transmission decreased with older age, after adjustment for viral load, and this decrease was significant for those who were 30 to 39 years of age (rate ratio, 0.32) and those who were 40 to 59 years of age (rate ratio, 0.27). The interaction between age and log-transformed viral load was not statistically significant (P=0.06). The risk of transmission was lower among circumcised male subjects than among uncircumcised male subjects, but this difference was not significant (rate ratio, 0.41; 95 percent confidence interval, 0.10 to 1.14).

The risk of infection increased as the HIV-1-infected partner's viral load increased and decreased with age among HIV-1-negative partners. The risk

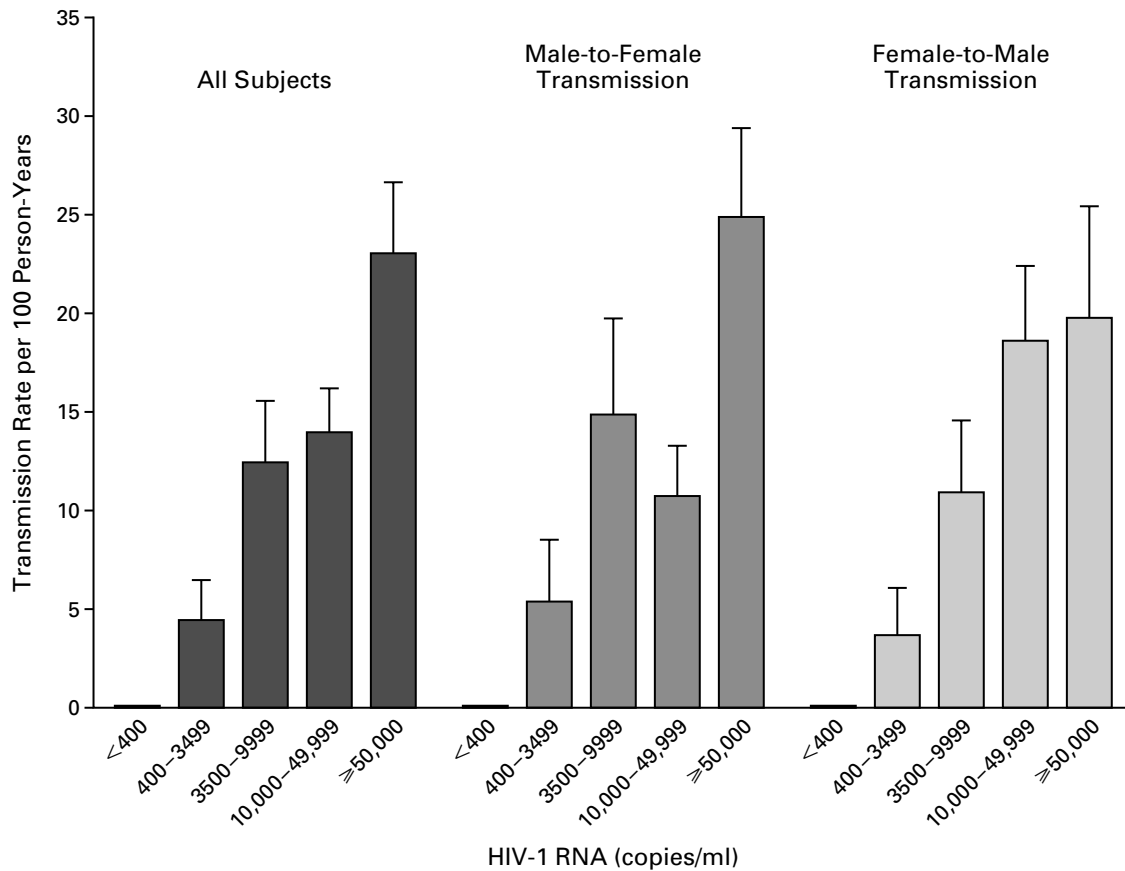


Figure 1. Mean (+SE) Rate of Heterosexual Transmission of HIV-1 among 415 Couples, According to the Sex and the Serum HIV-1 RNA Level of the HIV-1-Positive Partner.

At base line, among the 415 couples, 228 male partners and 187 female partners were HIV-1-positive. The limit of detection of the assay was 400 HIV-1 RNA copies per milliliter. For partners with fewer than 400 HIV-1 RNA copies per milliliter, there were zero transmissions.

of infection was zero among the 50 HIV-1-negative circumcised male subjects. A history of multiple sexual partners, symptoms of sexually transmitted diseases, or the laboratory diagnosis of sexually transmitted diseases had no significant effect on the risk of HIV-1 infection.

DISCUSSION

Prospective studies of HIV-1-discordant couples provide important information on the efficiency of transmission and the biologic and behavioral variables that influence the infectiousness of and susceptibility to HIV-1. Our study of heterosexual transmission among sexual partners was a community-based study in which all consenting couples, whether discordant for HIV-1 or not, were prospectively followed to evaluate the risk of transmission in relation to viral load and other characteristics. Our study sample is representative of the general population in this rural area of Uganda.

All participants were asked whether they wanted to know the results of their HIV-1 tests, all were offered counseling after testing and free condoms in the privacy of their own homes, and all were told about safe-sex practices. Couples counseling was also offered to the entire community, and all subjects were strongly encouraged to share the results of testing with their partners. Although the rate of condom use remained low in the entire study population, as has been the case in other studies in Uganda,²⁸ we did observe an increase in current condom use over the four-year study, from 4.4 percent to 7.4 percent as reported by women and from 9.9 percent to 16.9 percent as reported by men; these values represent some of the highest rates of use in rural sub-Saharan Africa. However, with this rate of condom use, HIV-1 was transmitted to 90 of the 415 initially HIV-1-negative partners, for an overall incidence of 11.8 per 100 person-years. This was significantly higher than the incidence of 1.0 per 100 person-years reported among

TABLE 4. ADJUSTED RATE RATIOS OF THE RISK OF TRANSMISSION AND ACQUISITION OF HIV-1.*

COVARIATE	RISK OF TRANSMISSION AMONG HIV-1-POSITIVE PARTNERS	RISK OF ACQUISITION AMONG HIV-1-NEGATIVE PARTNERS
	adjusted rate ratio (95% CI)	
Serum HIV-1 RNA level in HIV-1-positive partners		
<3500 copies/ml†	1.0	1.0
3500–9999 copies/ml	5.80 (2.26–17.80)	5.81 (2.25–17.91)
10,000–49,999 copies/ml	6.91 (2.96–20.15)	6.84 (2.93–19.97)
≥50,000 copies/ml	11.87 (5.02–34.88)	12.55 (5.28–36.99)
Level (per log increment)‡	2.45 (1.85–3.26)	2.45 (1.86–3.26)
Age		
15–19 yr†	1.0	1.0
20–29 yr	0.68 (0.32–1.61)	0.69 (0.36–1.42)
30–39 yr	0.32 (0.14–0.84)	0.38 (0.17–0.86)
40–59 yr	0.27 (0.10–0.81)	0.38 (0.17–0.88)
Female HIV-1-positive partner	0.76 (0.46–1.26)	0.77 (0.50–1.21)
Circumcision§	0.41 (0.10–1.14)	—
≥2 sexual partners in past yr (vs. 1)	1.16 (0.71–1.85)	0.69 (0.36–1.27)
Genital ulcer disease	0.93 (0.48–1.66)	0.77 (0.33–1.60)
Genital discharge	1.78 (0.86–3.45)	1.26 (0.51–2.79)
Dysuria	1.08 (0.53–2.05)	0.73 (0.31–1.49)
Syphilis	0.72 (0.34–1.32)	1.20 (0.59–2.23)
Gonorrhea¶	1.17 (0.23–3.83)	1.01 (0.86–1.19)
Chlamydia¶	0.22 (0.01–0.97)	—
Trichomonas	1.84 (0.84–3.89)	1.27 (0.65–2.35)
Bacterial vaginosis	0.91 (0.45–1.80)	0.77 (0.44–1.36)

*Each variable was adjusted for all the other variables. Poisson regression analysis was used to calculate the rate ratios. CI denotes confidence interval.

†These subjects served as the reference group.

‡The log (base 10) continuous model was constructed separately from the categorical estimates.

§A model was constructed that included only male subjects (228 HIV-1-positive and 187 HIV-1-negative subjects).

¶Estimates were based on a subgroup for which the results of a ligase chain reaction assay were available (199 HIV-1-positive subjects and 226 HIV-1-negative subjects).

||A model was constructed that included only female subjects (187 HIV-1-positive and 228 HIV-1-negative subjects).

couples in which both members were initially seronegative.²¹

The major finding of this study was the strong association between increasing serum HIV-1 RNA levels and an increasing risk of heterosexual transmission of HIV-1. In a finding similar to those of studies that found that the risk of perinatal HIV-1 infection is associated with the maternal viral load,^{24,29–32} we found a dose–response effect: the rate of transmission increased from 2.2 per 100 person-years to 23.0 per 100 person-years as the serum HIV-1 RNA level increased from less than 3500 copies per milliliter to 50,000 or more copies per milliliter (adjusted rate ratio, 11.87). In multivariate analyses, the serum HIV-1 RNA level was the main predictor of the risk of transmission (Table 4). Each log increase in viral load was associated with an increase by a factor of 2.45 in the risk of transmission. There were no instances of trans-

missions by seropositive subjects with undetectable viral loads or with serum HIV-1 RNA levels of less than 1500 copies per milliliter. This finding raises the possibility that reductions in viral load brought about by the use of antiretroviral drugs could potentially reduce the rate of transmission in this population. Such reductions in transmission have been documented in studies of perinatal transmission,^{30,32,33} but not in studies of sexual transmission. Further studies measuring the effects of antiretroviral drugs on sexual transmission are urgently needed.

Several studies have shown a good correlation between peripheral-blood viral load and viral load in seminal plasma³⁴ and cervical secretions,^{35,36} and viral loads in genital secretions appear to fall in concert with the declines in peripheral-blood viral load after combination therapy.^{20,34,37} However, the rate of transmission of HIV-1 was not assessed in these studies,

and despite reductions in peripheral-blood and seminal plasma viral load, integrated viral DNA is still present in seminal cells, and virus can be recovered in vitro.³⁸⁻⁴⁰ However, it is apparent from our results that the rate of transmission is markedly reduced among persons with very low serum viral loads.

In multivariate analyses, we did not find a significant association between the risk of HIV-1 transmission and the presence of sexually transmitted diseases or symptoms of sexually transmitted diseases in HIV-1-positive partners, or between an increased susceptibility to infection and sexually transmitted diseases among HIV-1-negative partners. However, genital discharge and dysuria in the seropositive partner were significant in the unadjusted analysis. This last finding, even though not significant in the multivariate analysis, is compatible with findings from other studies in which persons with a genital discharge had increased HIV-1 RNA levels in genital secretions.^{41,42}

In analyses of the risk of transmission according to male or female sex, we found no significant difference in incidence between female-to-male transmission and male-to-female transmission. The rate in each group was about 12 per 100 person-years. For each category of viral load, the rates of transmission were similar in both sexes, and these results reflect the nearly equal distribution of HIV-1 infection between men and women in this community and in most other parts of Africa.^{1,43} The transmission rates reported here reflect a combination of the probability of transmission per sexual act, the frequency of sexual contact, viral shedding in the genital tract as influenced by the presence of concurrent genital tract infections, and other variables.

Despite similarities in transmission rates between the sexes at each level of viral load, seropositive female subjects did have significantly lower log-transformed mean viral loads than male subjects, and this sex-specific difference was greatest among the subjects who transmitted the virus to their partners (mean log-transformed viral load, 4.30 among seropositive female subjects and 4.62 among seropositive male subjects; $P=0.015$). These data are consistent with recent reports that female subjects have lower viral loads than male subjects matched with them for age and CD4 count, despite the fact that they had similar rates of progression and similar decreases in the CD4 count.^{44,45} The mechanisms for these sex-based differences in viral load are unclear.

An additional finding in our study was that circumcision was protective against HIV-1 infection, with no infections occurring among 50 circumcised HIV-1-negative male subjects, as compared with 40 infections among 137 HIV-1-negative uncircumcised male subjects. This finding suggests that male circumcision may reduce the risk of acquisition at all HIV-1 RNA levels. Studies among truck drivers, persons attending sexually transmitted disease clinics, and prostitutes

and their clients in Africa have shown that the absence of circumcision among men increases their risk of heterosexual acquisition of HIV-1,^{9,11,46} potentially because of an association with an increased frequency of sexually transmitted diseases among uncircumcised men.¹¹ This association between male circumcision and a decreased risk of infection with HIV-1 may partially explain the low frequency of female-to-male transmission in U.S. studies of HIV-1-discordant couples,⁴⁷ since over 70 percent of men in the United States are circumcised.

Limitations in the interpretation of our data include the fact that the interval between the measurement of the viral load in the index subject and documentation of seroconversion in the partner was 10 months, resulting in some imprecision as to the viral load at the time of transmission. Similarly, the diagnosis of sexually transmitted diseases was established at the visit before and the visit after the end of the interval in which there was a risk of seroconversion, which may have diluted the potential association between sexually transmitted diseases and the risk of transmission of HIV-1. However, data on symptoms of sexually transmitted diseases were available for the entire interval in which there was a risk of seroconversion, and serum viral load was a much stronger predictor of the risk of transmission than was the presence of such symptoms in either partner.

Heterosexual transmission involves a complex interaction between biologic and behavioral factors. Our data suggest that peripheral-blood levels of HIV-1 RNA contribute dramatically to the risk of heterosexual transmission. Serum HIV-1 RNA levels below 1500 copies per milliliter were not associated with transmission, whereas the risk of transmission increased substantially with increasing viral loads. These results suggest that research is urgently needed to develop and evaluate cost-effective methods, such as effective and inexpensive antiretroviral therapy or vaccines, for reducing viral load in HIV-1-infected persons. Such measures, coupled with education about safe-sex practices, condom use, HIV-1 testing and counseling, and control of sexually transmitted diseases, could potentially reduce the infectivity of and susceptibility to HIV-1 and prevent further sexual transmission of the virus.

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