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HIV Incidence Rates and Risk Factors for Urban Women in Zambia: Preparing for a Microbicide Clinical Trial

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Abstract

Objectives—A preparedness study was conducted to evaluate the suitability of sites and populations following the same study procedures intended for a larger scale microbicide efficacy trial. In the process the study evaluated human immunodeficiency virus (HIV) incidence, prevalence, and risk profiles for HIV-acquisition among young women in urban Zambia.

Methods—Women aged 16 to 49 years were screened for participation in the study that involved HIV/sexually transmitted infection testing and the assessment of sexual behavioral characteristics. Two hundred thirty-nine eligible women were enrolled and followed up for 12 months.

Results—Baseline HIV prevalence at screening was 38.7% (95% CI: 34.2%–43.3%). The highest age-specific prevalence of HIV was 54.1% (95% CI: 46.3%–61.8%) seen in women aged 26 to 34 years. HIV incidence was 2.6% per 100 woman years. Pregnancy rates were high at 17.4 per 100 woman years (95% CI: 12.2–24.1).

Conclusion—It was concluded that our general population sample, characterized by high HIV prevalence and ongoing incidence rates despite receiving regular risk reduction counseling and free condoms qualifies for future microbicide studies.

A microbicide preparedness study conducted in Lusaka, Zambia found high HIV prevalence and appreciable HIV incidence in a population of women in an urban setting.

The zambia demographic and Health Survey of 2001–2002 estimated the prevalence of human immunodeficiency virus (HIV) among women aged 15 to 49 in Lusaka, Zambia at 22%.1 The survey estimated national syphilis prevalence at 6.5% among all women and 7.1% among pregnant women. Estimates of HIV incidence have ranged from 4.5 per 100 person years in an HIV prevention trial2 to 9.3 per 100 person years in a study of serodiscordant couples.3 National HIV programs have been established with emphasis on HIV treatment and prevention of mother-to-child transmission. Other prevention efforts have largely focused on promotion of abstinence, marital faithfulness, and condom use. However, several socioeconomic and cultural factors preclude many women from using these methods of HIV prevention effectively.

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Microbicide clinical trials are essential to evaluate the safety and effectiveness of vaginal products for use by women to prevent genital tract infections including HIV.4·5 HIV Prevention Trials Network (HPTN) 055 was a women's observational cohort study conducted to assess site suitability and preparedness for an expanded Phase II/IIb safety/ efficacy microbicide clinical trial (HPTN 035) in Lusaka, Zambia. HPTN 055 allowed evaluation of behavioral and biologic risk factors for baseline HIV infection and HIV seroconversion in those who were seronegative at enrollment. The study also evaluated sexually transmitted infections (STIs), reproductive tract infections (RTIs), and reasons for noneligibility for HPTN 055, using criteria that would be applied for the planned Phase II/ IIb microbicide trial. The study protocol targeted annual retention rates of 95%. This was to ensure that loss-to follow-up rates did not exceed the 5% to 6% average annual seroincidence rates that were expected to be observed in the control arms of HPTN 035 at the African sites. The projected annual incidence rate for Lusaka was 2.5 per 100 person years of follow-up.

METHODS

HPTN 055 methods and results have been presented in another publication for the 3 participating nations (South Africa, Tanzania, and Zambia).6 Detailed Zambia-specific data are presented here. Ethical approval was obtained from local and international ethics committees. Individual written informed consent was obtained separately for screening and enrollment. Enrolled women received regular HIV/STI risk reduction counseling, free basic medical care, condoms, and Pap smears. In addition, we provided a prepaid health scheme card that enabled participants to access medical services from government health centers outside of study clinic hours.

Population and Recruitment Strategies

HPTN 055 was conducted at 2 clinics located on the grounds of 2 Government-run health centers. Community sensitization involved drama and dance presentations, stakeholder and general community meetings, flyer distribution, door-to-door campaigns, and one-to-one discussions with potential participants. These strategies were targeted at populations of women aged 16 to 49 years and were conducted in the catchment area of the study clinics. It was anticipated that this specific reproductive age group would have significant HIV incidence. The recruitment message focused on the purpose of the study and the eligibility criteria. Sensitization talks were given in the Maternal Child Health and Family Planning clinics and the STI/Out-Patient Departments of the health centers. Non-study health workers in the health centers in which the study was being conducted and other surrounding health centers were sensitized and encouraged to refer eligible participants to the study. Interested women were invited to the study clinic where study staff provided more detailed information.

Screening and Enrollment

The first step to study screening was a urine hCG (human chorionic gonadotrophin) pregnancy test. Nonpregnant volunteers proceeded to pretest counseling and dual rapid venous HIV testing using both the Abbott Determine HIV 1/2 (Inverness Medical Innovations, Inc., Waltham, MA) and the OraSure OraQuick (OraSure Technologies, Inc., Bethlehem, PA) test kits. Blood was additionally drawn for syphilis rapid plasmin reagin (RPR) testing using Immutrep RPR Omegatest kits (Omega Diagnostics Ltd Alva FK 12, Scotland, UK). HSV-2 serology used HerpeSelect2ELISA IgG (Focus Diagnostic, Cypress, CA.). Pelvic examinations were conducted in all volunteers. Genital swabs were collected for detection of *Neisseria gonorrhoeae* by culture and Gram stain.

Chlamydia trachomatis was diagnosed using CHLAMY-FAST Solus (International Microbio, Signes, France). Wet prep microscopy with saline and potassium hydroxide was used to detect *Trichomonas vaginalis* and *Candida albicans*. Bacterial vaginosis was diagnosed by Amsel's criteria applied to a saline wet preparation.

Treatment of STIs and other reproductive tract infections was based on the 2003 World Health Organization Guidelines for the Management of STIs. Volunteers who were free of STIs/RTIs and met protocol eligibility criteria were eligible for study enrollment.

Study Participant Follow-Up

Duration of participant follow-up was 1 year with monthly study visits. HIV, syphilis, and STI testing with pelvic exams were done quarterly per protocol specifications. Questionnaires administered quarterly assessed HIV/STI risk behavior and vaginal hygiene practices. At all visits, study participants received HIV/STI risk reduction counseling, and male latex condoms were provided. Retention activities involved collection of comprehensive locator information and prompt follow-up of missed visits.

Statistical Analysis

Methods used for statistical analysis for the Lusaka subgroup were identical to those used for cross-site analysis as reported by Ramjee et al.6 Descriptive statistics summarized demographic and sexual behavior characteristics. Changes from baseline to follow-up were analyzed via McNemar's test for dichotomous variables and a test or Wilcoxon signed rank test (as appropriate) for continuous variables. CIs were based on the binomial distribution for prevalence rates and the Poisson distribution for incidence rates.

RESULTS

HIV Prevalence and Reasons for Study Ineligibility

A total of 542 women were screened for interest and eligibility in HPTN 055. Because screening was a multistep process, potential participants were eliminated at various stages of the screening process. Of the women screened but not enrolled, 87 were not tested for HIV. Of 455 who underwent HIV testing, 176 tested HIV positive (38.7%, 95% CI: 34.2%–43.3%), making HIV infection at baseline the most common reason for ineligibility. Prevalence was highest in the 25 to 34 year age group at 54.1% (95% CI: 46.3%–61.8%). Significant risk factors for HIV infection on a multivariable logistic regression analysis were an older mean age (P < 0.01) and reporting that a woman earned her own income (P = 0.02, Table 1).

Other reasons for ineligibility included all screening procedures not completed in the 30-day screening window (38/542, 7%); not sexually active as defined by 1 vaginal sex act in last 3 months (31/542, 6%); currently pregnant (19/542, 3.8%); unable or unwilling to provide informed consent (14/542, 3%). Among all women screened, 239 (44.1%) were enrolled over a span of 7 months.

Prevalence of RTIs, STIs, and Pregnancy

The prevalence rates of STIs, RTIs and pregnancy at the time of screening are illustrated in Table 2. Syphilis, vaginal candidiasis, and HSV-2 were particularly prevalent. The other STIs gonorrhoea, chlamydia, and trichomoniasis were less commonly seen.

Behavioral and Reproductive Health Characteristics of Enrollees

A total of 237 women out of 239 (99%) reported having had only 1 sex partner in the 3 months before enrollment; 2 women reported having 2 partners, and no women reported more than 2 partners. Of the women who had vaginal sex in the week before enrollment, 98 of 162 (60%) reported condom use. Nearly half of the women (111/239, 46%) reported douching before sex at the time of the last vaginal intercourse (Table 3), whereas 183 of 239 (77%) had douched after their last vaginal intercourse. About one-third (81/239) inserted cloth or cotton wool with the last vaginal intercourse and 8 of 239 (3%) inserted other objects after the last vaginal sex. Five percent (13/239) of enrolled women reported inserting medicines and/or herbs in the vagina in the month before enrollment. Anal sex was reported rarely (2/239, <1%).

Family planning methods were not mutually exclusive. Male condoms were reported as a current family planning approach by 56% (133/239) of enrolled women. Oral contraceptive pills were used by 23% (56/239) and injectable contraceptives were used by 20% (47/239). About 1 in 5 women reported using unspecified "natural methods" of birth control (21%, 49/239) and 11% (27/239) of women reported not using any form of birth control.

Behavioral Assessment in Follow-Up

We compared sexual behavior at enrollment and at month 12 to assess the impact of our educational interventions (Table 3).

A significant decline was noted in the number of women who douched before sex and in those who reported using natural family planning, both of which had been discouraged. The use of injectable contraceptives increased significantly. In contrast, no statistically significant changes were noted in vaginal sex without a condom in the preceding 3 months or in douching after sex.

Incidence Rates for HIV, STIs, and RTIs

Six of 239 women enrolled (2.6/100 woman-years; 95% CI: 1.0-5.7) became HIV infected during the 1-year follow-up period (Table 4). STI/RTI acquisition was common over the 12 months. Vaginal candidiasis, bacterial vaginosis, and trichomoniasis had especially high incidence rates.

Pregnancy Incidence

Despite family planning counseling and condom distribution, 36 participants became pregnant during the study, a pregnancy incidence rate of 17.4 per 100 woman-years (95% CI: 12.2–24.1). The incidence rate was 24.1, 12.7, and 9.6 per 100 woman-years in the 18 to 24, 25 to 34, and \geq 35 age groups, respectively. Pregnancy incidence rates were also calculated stratifying by contraceptive method reported at baseline. The highest pregnancy incidence rate was observed among women who reported using no contraceptive at baseline (29.4/100 woman years; 95% CI: 10.8 – 64.0) and the second highest was in women who reported using female condoms at baseline (23.3/100 woman years; 95% CI: 2.8 – 84.1). Pregnancy incidence was lowest (11.9/100 woman years 95% CI: 4.4 –25.9) in those who had reported use of contraceptive pills.

Retention

Monthly retention was at least 90% for all months and 93% of participants were retained at the month 12 visit.

DISCUSSION

True incidence data for HIV, STIs, and pregnancy are invaluable for assessing the impact of interventions, planning clinical trials, and assessing HIV "hot-spots" of current transmission. High seroincidence rates despite continual risk reduction counseling and the provision of free condoms throughout study duration were most concerning. A condom promotion study conducted in Zimbabwe and Malawi reported similar findings.7 Our study participants were not specifically recruited from high-risk venues.

Marital status did not show a statistically significant association with HIV-positive status, although being married has been identified as a significant predictor of HIV seropositivity in other studies.8^{,9} Having a source of independent income was also found to be a risk factor for HIV. Independent income may be a marker of women who are in jobs that subject them to sexual harassment, entail sexually-related income generation, involve alcohol consumption, or exposure to wider or riskier social networks.10

The prevalence of both HIV and syphilis among women screened was higher than the prevalence among antenatal attendees in Zambia in 2004 that was 18.7% and 6.9% for HIV and syphilis, respectively.11 Site staff believed a significant proportion of women who volunteered for screening for HPTN 055 were high-risk women who suspected they were HIV-infected and used the screening process to confirm their status. In addition, community sensitization carried the message about free STI screening and treatment services, such that persons who had STI symptoms or who considered themselves to be at risk were more likely to present themselves to the clinics for screening. Over one-third of women screened tested positive for HSV-2 infection, a known predisposing factor for HIV infection.12,13 HIV Prevention Trial Network (HPTN 039) reported an HSV-2 prevalence of 50% among HIV negative women in Lusaka (Stewart Reid, HPTN 039 Investigator of Record, Lusaka, Zambia, personal communication). This study targeted women who experienced episodic genital itching or genital ulceration and were, therefore, more likely be HSV-2 positive. A prevalence of 55% was found by Weiss et al in a population of women in another Zambian city.14 Different socioeconomic factors could account for the variation in prevalence in comparison to our population. One of the study objectives was to assess behavior at enrollment and at 1-year follow-up to identify behavioral risk factors for HIV acquisition; however HIV incidence was too low to permit confidence in our results. Nonetheless the incidence rate in the 18 to 24 year age group was significantly higher than the overall incidence rate suggesting that the most risky sexual behavior occurred in this age group. Given the high HIV prevalence of screened women at baseline, enrolled participants above the age of 24 may only represent women who were at lower risk. The mean number of sex partners essentially remained unchanged suggesting that a woman's risk may in part be derived from her partner's behavior or other external factors.15

The study offered condoms and risk reduction messages to participants at every study visit. However, condom use rates did not significantly change from enrollment to study completion one year later. Findings that condom use did not improve despite our persistent risk reduction messages and readily available condom supplies may imply that women are not the sexual decision makers in relationships.16 Alternatively condom use may not be the preferred HIV/STI prevention strategy by women themselves. Further research is required to clarify women's preferences. However, the high proportion of married women included in this population may have affected condom use rates as married women are less likely to use condoms than unmarried women.17¹8

Condom use was higher than that noted in the 2001–2002 Zambia Demographic and Health Survey that estimated overall condom use with the last intercourse to be 11.8% in an

interview of Zambian women.1 Since the women in HPTN 055 had similar condom use risk characteristics from screening to month 12, it is suggested that women who have been in a preparedness study for as long a year still remain at risk, and could be suitable for participation in Phase 2 or 3 prevention clinical trials.

The practice of vaginal douching is widespread in Zambia as in other African societies.19^{,20} Douching before sex appeared to have declined during the study. Competing explanations are that women were responsive to advice to stop douching21 or that responses were influenced by a social response bias.

The observed increase in uptake of injectable family planning and the reduction in use of "natural methods" could be attributed to the ready availability of family planning services on site and to regular contraceptive counseling. The high overall pregnancy incidence rate reflects the challenge of recruitment into microbicide clinical trials of young sexually active women who are likely to become pregnant as a result of partner or peer pressure or of their own desire. In this study, women in the 18 to 24 year age group were most prone to pregnancy. The resultant temporary hold of study product use during pregnancy diminishes the number of woman years accrued for final study analysis. The high pregnancy rate obviously indicates continued unprotected sexual activity by study participants.

The study's failure to increase condom use but the apparent success in reducing women's douching/vaginal product insertion and increasing use of family planning trends underscore the need for prevention strategies that may be easier to negotiate with male partners. It also reinforces the need for couples counseling, to engage men in the educational process.22·23 This finding further highlights the beneficial role of a truly female controlled prevention method that does not require partner cooperation.

A limitation of the study is that it did not use an intravaginal product that might have better prepared our communities for the microbicide trial to come. Thus the impact of study product use on enrollment and retention as well as microbicide acceptability could not be assessed in this preparedness study. Strengths of the study included obtaining cohort incidence data while offering risk reduction counseling and VCT, mimicking the microbicide trial itself, as well as our assessment of STI incidence rates.

In conclusion, we found an exceedingly high HIV prevalence, at baseline for screened women (38.7%); continuing risk for HIV infection for seronegative women enrolled (2.6 per 100 person-years); high STI and pregnancy rates; and a high frequency of vaginal hygiene practices such as douching. All belie the seriousness of the southern African HIV epidemic. These factors also qualify this population for future microbicide studies for which our data suggest the following:

Younger women between the ages of 18 and 24 would be the ideal candidates for future microbicide studies as evidenced by the high seroconversion rate in this age group.

Reliable contraception must be aggressively marketed and provided on site both to meet the unmet need and also to ensure the integrity of the trial.

Microbicide trials are feasible in Zambia. In addition risk reduction counseling must be an essential component for an ethical and successful HIV prevention clinical trial.

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TABLE 1

Comparison of Demographic Characteristics of HIV Negative and Positive Women at Screening

	HIV Status	at Screening	
Demographic Characteristic	HIV Positive (%)	HIV Negative (%)	Р
No. Women	176	279	
Mean age in years (±SD)	27.9 (6.0)	26.7 (7.9)	< 0.01
Married	130 (74%)	234 (71%)	0.52
Husband known to have >1 wife	22 (13%)	28 (10%)	0.44
Currently living with husband or partner	129 (75%)	192 (71%)	0.39
At least some secondary school education	92 (52%)	155 (56%)	0.50
Woman earns own income	76 (43%)	90 (32%)	0.02
Partner earns at least some income	148 (86%)	233 (86%)	0.99

TABLE 2

Prevalence Rates of STIs, RTIs, and Pregnancy for Women at Screening

	#No. Tested	No. Positive	%	95% CI
Neisseria gonorrhoeae	265	9	3.4	1.6-6.3
Chlamydia trachomatis	263	7	2.7	1.1-5.4
Syphilis	454	57	12.6	9.6–16.0
Bacterial vaginosis	244	16	6.6	3.8-10.4
Trichomonas vaginalis	244	9	3.7	1.7–6.9
Candida spp.	244	46	18.9	14.1-24.3
HSV-2	239	93	38.9	32.7-45.4
Pregnancy	475	18	3.8	2.3-5.9

TABLE 3

Sexual Behavior at Enrollment and at Month 12 (n = 239)

Sexual Behavior	Enrollment (n = 239)	Month 12 (n = 223)	р
Mean no. sex partners, past 3 mo (±SD)	1.0 (0.1)	0.9 (0.3)	< 0.01
Vaginal sex without condom, past 3 mo	193 (81%)	166/199 (83%)	0.65
Mean no. condom uses with vaginal sex, past 1 week (\pm SD)	1.1 (1.3)	0.9 (1.1)	< 0.01
With last vaginal sex: douched before sex	111 (46%)	64/199 (32%)	< 0.01
With last vaginal sex: douched after sex	183 (77%)	143/199 (72%)	0.10
With last vaginal sex: inserted cloth or cotton wool	81 (34%)	64/199 (32%)	0.57
Family planning using "natural methods"	49 (21%)	14 (6%)	< 0.01
Current family planning: oral contraceptive pills	56 (23%)	54 (24%)	0.88
Current family planning: injectables	47 (20%)	64 (29%)	< 0.01
Current family planning: norplant	5 (2%)	7 (3%)	0.16

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HIV and STI/RTI Incidence Stratified by Age Group

			Incidence Rate By Age Group (yrs)	ite By Age G	roup (yrs
	Total New Infections or Events Incidence Rate Per 100 PY	Incidence Rate Per 100 PY	18-24 25-34	25–34	353
HIV	9	2.6	4.7	0	2.2
Neisseria gonorrhoeae	11	5.1	5.8	3.2	4.6
Chlamydia trachomatis	5	2.3	1.9	4.7	0
Syphilis	5	2.3	2.0	4.8	0
Bacterial vaginosis	25	12.8	15.8	10.1	7.4
Trichomonas vaginalis	14	6.9	9.6	0	9.8
Candida spp.	73	50.5	57.4	41.3	40.3
HSV-2	Not done				