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Original Research Article

Factors Associated with HIV Prevalence in a Pre-Partum Cohort of Zambian Women

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Summary: An ongoing study of mother-to-child human transmission in Zambian women ($n = 3,160$) allowed us to examine the association of medical injections with HIV serostatus while simultaneously accounting for other factors known to be correlated with HIV prevalence. Multi-method data collection included structured interviews, medical record abstraction, clinical examinations, and biological measures. Medically administered intramuscular or intravenous injections in the past five years (but not blood transfusions) were overwhelmingly correlated with HIV prevalence, exceeding the contribution of sexual behaviors in a multivariable logistic regression. Statistically significant associations with HIV also were found for some demographic variables, sexual behaviors, alcohol use, and sexually transmitted diseases (STD). The results confirmed that iatrogenic needle exposure, sexual behavior, demographic factors, substance use, and STD history are all implicated in Zambian women's HIV + status. However, the disproportionate association of medical injection history with HIV highlights the need to investigate further and prospectively the role of health-care injection in sub-Saharan Africa's HIV epidemic.

Keywords: medical injections and HIV, Zambian women and HIV, association with HIV prevalence.

Introduction

HIV/AIDS remains the leading cause of mortality in sub-Saharan Africa^{1,2} where more than 90% of the HIV infections are believed to result from heterosexual transmission.^{3,4} Recently, Gisselquist and his colleagues⁵ expressed skepticism that heterosexual transmission alone can explain the magnitude of sub-Saharan Africa's HIV/AIDS pandemic. Contrary to prevailing opinions, they estimated that only 25-29% of the sub-Saharan HIV infections in women and 30-35% in men result from sexual transmission and suggested that needle exposures to HIV during health-care encounters may account for more HIV infections in sub-Saharan Africa than do sexual exposures.^{6,7} Several later articles presented mathematical models empirically demonstrating an unexplained gap in the available empirical data that was not explained by sexual behavior.^{8,9} In these articles, the authors disputed the logic of continuing to rely on sexual transmission alone to explain HIV's spread across the continent and suggested that medical injections were likely to be the missing explanation for the disparity revealed by the mathematical models. Initially, the literature addressing

this possible discrepancy was largely theoretical, based on literature reviews, rational discourse and mathematical modeling. The plausibility of this assertion has been questioned,¹⁰ but very recent converging evidence using a variety of methodologies and research methods is producing convergent evidence that more careful attention needs to be directed to the role of medical injections as a contributor to the spread of this pandemic.^{11,12}

Our research team has accumulated a large data set from an ongoing longitudinal study of Zambian prepartum women that allowed us to examine the association of medical injections with HIV prevalence while controlling for demographic characteristics, sexual behaviors, substance use, cultural practices, and sexually transmitted diseases (STD) already known to have an association with HIV infection.

Method

Baseline data were collected from 3,160 prepartum Zambian women at University Teaching Hospital (UTH) affiliated with the University of Zambia School of Medicine, the largest tertiary care setting in Zambia from

1989-2001. Participants had been recruited into a longitudinal study on mother-to-child transmission of Kaposi's sarcoma-associated herpes virus infection (HHV-8, also known as KSHV). Inclusion criteria included: (a) resident of Lusaka; (b) not in active labor, and (c) without any clinically diagnosed diseases that affect the immune system (AIDS, tuberculosis, cancer, Kaposi's sarcoma (KS), or malaria). Self reported medical histories, medical record examination, and clinical evaluations ruled out any past or current indications of these diseases. All participants were informed about the purposes of the primary study, provided informed consent, and were counselled by certified counsellors.

Data collection methods

Following informed consent, participants received clinical examinations by participating physicians and completed a standardized interview. Medical history data were abstracted, with the patients' permission, from hospital charts. All women were tested for HIV, syphilis, and HHV-8. Biological tests were processed in the UTH lab and are described below.

Variables utilized for the present analysis were identified from a literature review as having an association with HIV and then organized into six blocks, each with multiple variables: demographic information (age, tribal membership, education, religion, marital status, and employment), medical injections (medically administered intramuscular (IM) or intravenous (IV) injections in past five years and past blood transfusions), self-reported sexual behavior (partner status, number of new partners in past three years, intercourse frequency, sex during menstruation, oral intercourse, anal intercourse, history of rape, sex partner with penile sores, condom use with primary partner, condom use with other partners, and condom breakages), alcohol or other substance use (alcohol use, drug use, condom use during intercourse while high) and indigenous cultural practices (use of vaginal drying agents, traditional scarification, and engaging in dry sex). All of these variables (apart from medical injections) previously were identified in other studies as having an association with HIV serostatus, but have never been incorporated into a single data set that could assess their relative associations with HIV prevalence simultaneously.

Biological test procedures

Plasma was tested for HHV-8 antibodies by indirect immunofluorescent assay (IFA), following the procedures described by Lennette *et al.*¹³ To exclude false positive results due to background staining, all positive plasma were tested in parallel with BJAB cells (HHV-8 negative B lymphoma cell line). Tests for HIV used two rapid assays, Capillus (Cambridge Biotech, Ireland) and Deter-

mine (Abbott Laboratories, USA) following the manufacturer's recommended procedures. Plasma that tested positive on a Capillus assay was confirmed by a Determine assay. Syphilis serology was tested with the rapid plasma regain (RPR) assay (Arlington Scientific, Inc., Springville, Utah) and a *Treponema pallidum* hemagglutination assay using the Serodia®-TPHA kit (Fujirebio, Inc., Tokyo, Japan). Syphilis was considered present if both assays were reactive. Women who reported syphilis infection that was treated and no longer evident on the biological measures were also coded as having had syphilis in the past.

Statistical analyses

Data analysis used the Statistical Package for the Social Sciences version 12.0 (SPSS-12). Frequencies were computed for the categorical variables and means for the continuous variables. Significance levels, odds ratios, and 95% confidence intervals were computed using multivariable logistic regression analysis. Confidence intervals were based on coefficients and standard errors in the logistic regression, as recommended by Hosmer and Lemeshow.¹⁴ Each cluster of variables was entered as a stepwise block into the regression. The HosmerLemeshow goodness-of-fit X^2 -test¹⁴ estimated the proportion of cases that could be correctly classified based on the measured variables.

Results

Sample

During the enrolment period, 92% of women receiving prenatal care from the UTH labor ward were screened for study inclusion and 3,470 women met the inclusion criteria. From these, 8.9% ($n = 310$) declined to participate for reasons that included 'need to consult husband,' 'not interested,' 'live too far away (far return visits),' 'involved in other studies,' 'do not want to know test results,' 'fear blood drawing,' and 'cannot afford to return for study follow-up visits.' The final sample included 3,160 women.

Most (98.6%) of the women were Zambian and the balance were from six other sub-Saharan countries. The Congo and Zimbabwe had the largest representation of other nationalities at 15 and 19 individuals, respectively. The sample included eight major Zambian ethnic groups as well as 29 additional minority ethnic groups that were categorized as 'other ethnicity' and together represented 3.4% of the sample.

Mean age of the sample was 24 years, 90.3% were married and 44.8% had completed some education beyond primary school. Median household income was the equivalent of US \$20-50/month and supported an average of 4.7 household members (range 1-25). Only 15.4%

of the women were employed in formal or informal positions and 84.6% were unemployed. Mean age at the time of sexual debut was 17.5 and 87.8% were currently living with a primary sex partner. Most (89.0%) reported no other sex partners within the past three years and only 1.3% of the sample reported having three or more sex partners within same period.

Within this sample of clinically healthy prepartum women with no clinical, self report, or medical record evidence of immune compromise, 30.3% were found to be HIV seropositive ($n = 956$).

Logistic regression analysis assessing the relationships of variable blocks and individual predictors within those blocks to HIV status

Clusters associated with HIV prevalence

As shown in Table 1, five of the six blocks in the logistic regression analysis (demographics, sexual behavior, alcohol and substance use, iatrogenic needle exposure, and STDs) showed a significant relationship to HIV seropositivity. The logistic regression used a stepwise entry for each block of variables, following the order in which each block is listed below and as presented in Table 1. As shown in Table 1, after controlling for demographic variables, sexual behavior, and substance use, iatrogenic needle exposure added substantively to the model with a X^2 value of 105.79. As a check on the model, additional logistic regression analyses were conducted varying the order of entry to assess whether entry order altered the findings in any way. None of those precautionary checks altered the domains or the variables within those domains that emerged as significant in the primary stepwise logistic regression.

Variables within the significant blocks that predicted HIV infection

Table 2 presents the beta value, standard error, significance level, odds ratio, and 95% confidence interval for each variable and for the categories within variables for

Table 1 Omnibus tests of model coefficients: blocks that predicted HIV infection in logistic regression analysis

Domain	χ^2	df	Significance ($P <$)
Demographics	54.88	21	0.0001
Sexual behaviour	66.30	18	0.0001
Alcohol and substance use	22.83	3	0.0001
Iatrogenic needle exposure	105.79	2	0.0001
Cultural practices	0.27	3	0.97
STDs	14.29	7	0.05

Note: significant associations are shown in bold face type.

the measures included in the significant blocks. Statistically significant variables and categories are displayed on the table in bold face type.

Demographic variables associated with HIV Prevalence

Age, ethnicity, and employment status emerged as significant demographic predictors, while religious affiliation, educational level, and marital status showed no relationship to HIV prevalence. Not surprisingly, increasing age was associated with a greater probability of HIV infection. Membership in some ethnic groups was associated with significantly greater odds of being HIV seropositive. Finally, formal employment was protective. Women who were employed in formal positions were only half as likely to be HIV + as women who were in the informal sector or who were unemployed.

Sexual behaviors associated with HIV Infection Four of the 11 sexual behavior measures (intercourse frequency, oral intercourse, past rape, and condom use with primary partner) were significantly associated with the probability of being HIV seropositive. Currently living with a sex partner, number of new partners in the past three years, intercourse during menstruation, anal intercourse, having a sex partner with penile sores, condom use with non-primary partners, and number of reported condom breakages did not emerge as significant predictors. Counter-intuitively, the four significant sexual variables were protective. Women who engaged in intercourse more frequently, oral sex at least occasionally, experienced a rape in the past, and used condoms at least occasionally with their primary partner were less likely to be HIV +.

Alcohol and substance use Although the composite showed a significant relationship with HIV status as a block, individually none of the three variables included in this domain (alcohol use, drug use, and the percent that used condoms for sex when they were under the influence of alcohol) attained statistical significance. Two of these variables were infrequent within the sample as less than 1% of the sample reported any drug use and only 12% reported any alcohol use.

Iatrogenic needle exposure Even after accounting for the proportion of HIV infections associated with the domains listed above, medical injections emerged as the single most powerful predictor of HIV seropositivity. As shown in Table 2, this domain included two variables: having received an IM/IV injection in the past five years and having received a blood transfusion in the woman's lifetime. Past blood transfusion was infrequent, only 3% of the sample, and did not emerge as a significant predictor. However, having received a medically adminis-

Table 2 Individual variables within the significant clusters that predicted HIV infection

	Means/percentages	β -value	SE	<i>P</i> <	Odds ratio	95% CI
<i>Variables within blocks</i>						
Block 1: demographic variables						
Age	24.3 (Range 13–45)	0.02	0.008	0.005	1.02	1.01–1.04
Ethnic group				0.0001		
Bemba	30.2%	0.77	0.22	0.0001	2.17	1.4–3.3
Nyanga	44.0%	0.52	0.21	0.02	1.69	1.1–2.5
Tonga	13.2%	0.38	0.23	0.10	1.46	0.93–2.3
Lozi	4.6%	0.70	0.28	0.02	2.00	1.16–3.49
Kaonde	2.0%	0.74	0.36	0.04	2.10	1.04–4.23
Lunda	1.2%	1.52	0.52	0.004	4.58	1.64–12.78
Luvale	1.3%	–0.10	0.38	0.78	0.90	0.43–1.88
Other (ref)	3.4%					
Religion				0.63		
Traditional	0.4%	0.86	0.95	0.37	2.33	0.37–15.06
Christian	98.3%	0.60	0.66	0.36	1.82	0.50–6.61
Hindu	0.5%	1.62	1.04	0.12	5.04	0.66–38.63
Moslem	0.4%	0.51	0.93	0.59	1.66	0.27–10.23
Other (ref)	0.4%					
<i>Variables within domains</i>						
Education				0.78		
None	6.8%	–0.04	0.26	0.88	0.96	0.58–1.60
Primary (1–7 years)	48.4%	–0.17	0.21	0.40	0.84	0.56–1.26
More than primary (8–12 years)	39.3%	–0.16	0.20	0.43	0.85	0.57–1.27
More than secondary (ref)	5.5%					
Marital status				0.14		
Single	8.2%	0.27	0.61	0.65	1.32	0.40–4.34
Married	89.2%	–0.77	0.76	0.31	0.35	0.10–2.05
Divorced, separated	0.7%	–0.32	0.84	0.70	0.72	0.14–3.74
Widowed	0.4%	0.40	0.62	0.53	1.49	0.44–5.03
Other (ref)	0.4%					
Employment status				0.001		
Formal employment	8.3%	–0.65	0.18	0.0001	0.52	0.37–0.73
Informal employment	9.0%	–0.16	0.15	0.30	0.85	0.63–1.07
Unemployed (ref)	84.6%					
Block 2: sexual behaviour						
Currently living with sex partner, % yes	87.6%	0.10	0.15	0.47	1.11	0.84–1.47
Number of new sex partners, past 3 years	0.18 (range 0–8)	–0.04	0.08	0.66	0.97	0.83–1.13
Sexual intercourse frequency				0.03		
Daily	3.8%	–0.97	0.38	0.02	0.38	0.18–0.81
Several times/week	66.8%	–0.27	0.34	0.42	0.77	0.39–1.48
Once/week	17.1%	–0.42	0.33	0.20	0.66	0.34–1.26
Several times/month	10.1%	–0.38	0.34	0.27	0.69	0.35–1.35
Several times/year (ref)	2.2%					
Intercourse during menstruation, % yes	5.3%	–0.25	0.18	0.17	0.78	0.55–1.11
Engage in oral intercourse, % yes	5.5%	–0.48	0.18	0.01	0.62	0.44–0.87
Engage in anal intercourse, % yes	0.5%	–0.57	0.59	0.33	0.56	0.18–1.79
Rape victim, % yes	7.3%	–0.40	0.16	0.01	0.67	0.49–0.91
Sex partner with penile sores, % yes	5.6%	0.07	0.19	0.72	1.07	0.74–1.54
Condom use with primary partner				0.0001		
Always	0.4%	–0.95	0.62	0.12	0.39	0.12–1.30
Frequently	3.6%	–0.14	0.24	0.45	0.87	0.55–1.39
Occasionally	16.2%	–0.50	0.12	0.0001	0.61	0.48–0.77
Never (ref)	79.7%					
Condom use with other partner(s)				0.63		
Always	0.5%	–0.65	0.54	0.23	0.52	0.18–1.50

continued

Table 2 (Continued.)

	Means/percentages	β -value	SE	<i>P</i> <	Odds ratio	95% CI
<i>Variables within blocks</i>						
Frequently	0.9%	-0.27	0.39	0.59	0.76	0.29-2.00
Occasionally	2.7%	-0.09	0.27	0.76	0.92	0.54-1.57
Never (ref)	95.9%					
Number of condom breakages experienced	0.04	0.00	0.14	0.99	1.00	0.76-1.32
Block 3: alcohol and substance use						
Alcohol use, % yes	12.4%	-0.02	0.13	0.86	0.98	0.76-1.26
Drug use, % yes	0.9%	5.62	4.19	0.18	274.2	0.08-100181.5
Use condoms for sex when intoxicated, % yes	99.9%	-0.35	1.28	0.79	0.71	0.06-8.66
Block 4 iatrogenic needle exposure (ref=none)						
IM/IV injection in past 5 years, % yes	63.1%	0.95	0.10	0.0001	2.59	2.15-3.11
Blood transfusion (lifetime), % yes	3.0%	0.08	0.27	0.85	1.09	0.64-1.86
Block 5: cultural practices (ref=no)						
Traditional tx. with vaginal herbs, % yes	12.0%	0.05	0.16	0.75	1.05	0.78-1.42
Dry sex, % yes	17.5%	-0.05	0.11	0.65	0.95	0.77-1.18
Traditional scarification, % yes	26.4%	-0.01	0.13	0.92	0.99	0.76-1.28
Block 6: presence of other STDs (ref = no)						
Syphilis, % yes	8.2%	-0.29	0.17	0.08	0.75	0.54-1.04
Gonorrhoea	2.1%	-0.12	0.32	0.71	0.71	0.48-1.65
Chancroid, % yes	0.3%	-1.92	0.92	0.04	0.15	0.02-0.88
Genital herpes, % yes	1.2%	0.35	0.41	0.39	1.41	0.64-3.12
Genital warts, % yes	6.4%	-0.23	0.18	0.21	0.80	0.56-1.14
Bola bola, % yes	0.7%	0.34	0.59	0.57	1.40	0.44-4.47
HHV-8, % yes	39.9%	-0.16	0.09	0.09	0.85	0.71-1.02

Note: significant variables and significant categories within variables are shown in bold face type
SE = standard error; CI = confidence interval

tered injection within the past five years showed a strong association with prevalent HIV infection. Within the sample, 63.1% of the women had received an IV or IM injection during that interval and this was associated with a 2.59-fold greater likelihood of being HIV +.

Sexually transmitted diseases STDs in combination evidenced a significant relationship with HIV status. However, only chancroid attained individual significance within the cluster. Chancroid affected only eight women within this sample of more than 3000 and the finding may not be replicable given the exceedingly small number of women reporting past chancroid and reliance on self report for all STDs other than syphilis and HIV.

Goodness-of-fit and model prediction accuracy

Based on the variables included in the regression model, 72.0% of the sample could be classified into their correct HIV-status. Prediction was far more accurate for HIV- women at 95.8% correct classification than for HIV + women.

Discussion

Two major findings emerged from this exploratory study using an existing data set. Medical injection history made an overwhelming contribution to explaining prevalent HIV-infection, even after demographic variables, sexual behaviors, and substance use were already parcelled out of the variance in this regression equation. Clearly, when the current debate about the possible relationship of medical injections to HIV infection was applied to a real world data-set, medical injections were a substantial predictor of HIV prevalence. A second surprising finding was that more than 30% of this healthy cohort was already HIV +, despite being specifically recruited into the study because there was no archival or clinical evidence of any immune system compromise. In part, this may be due to referrals of higher risk women into UTH since the women who deliver at UTH include high-risk obstetric referrals. Another counter-intuitive result was that higher frequency and greater variations of sexual behavior were protective, rather than a risk factor for this cohort of largely monogamous women.

Measurement was limited to self-reports of having re-

ceived IV and IM medical injections in the past five years and past blood transfusion. Had dental procedures and other medical procedures that involve puncturing been included, there may have been an even stronger association. Another possibility that must be considered is that having received medically-administered injections could be a proxy for declining health, even though women with any evidence of compromised health using multiple indicators were ineligible for study participation. Mathematical modelling also found that this type of confound between health and needle exposure does not explain the proportion of HIV infections attributable to needle exposure.⁷

The greatest strength of this study is also its major limitation. This study's strength is that it was derived from an existing data-set collected for another ongoing study and was never intended to address the research question described in this analysis. Data collection was free of any experimental bias with respect to the current controversy over the relative importance of sexual behavior versus medical injections in the sub-Saharan pandemic. Its limitations are that this is a cross-sectional data set that cannot identify the reasons for medical injections, providers who administered them, or the settings in which they were delivered. More precise information is needed in future prospective research to clarify this issue. In addition, because most of this sample was monogamous and reported only one sex partner, it would add to our understanding of the transmission dynamics if both partners' in the marital dyads were included since it is plausible that this sample's primary sexual risk derived from the behavior of their partners.

The finding that increasing age was associated with a greater likelihood of infection is intuitive since the elapsed time since sexual debut allows for more sexual exposures. The variation in risk between different ethnic groups appears to be related to circumcision practices since groups at highest risk were those that do not routinely practice circumcision.¹⁵ Finally, formal employment had a protective effect for the women in this study. One can speculate that this may be because the independent income, sense of greater worth, and exposure to a wider range of people and views may better enable these women to behave in self-protective ways and reduce their reliance on a spouse for material needs.¹⁶

Most studies in sub-Saharan Africa emphasize the importance of sexual behavior in HIV transmission and acquisition¹⁷. Our results suggest that intercourse frequency, engaging in oral sex, a past rape, and at least occasional condom use with the primary (which in most cases meant 'marital') partner were protective and inversely related to HIV infection. While this may initially seem to be a surprising finding, it does make interpretive sense. There may be a long-term health benefit to women from more frequency and greater sexual variety within the marital dyad if these lead to greater sexual satisfac-

tion and fewer outside partners for the male spouse. While this cannot be established conclusively from the present study since it was limited to women only, it is consistent with other literature on risk and protective factors.¹⁸ The finding that even occasional condom use with main partners was protective is not surprising. The sheer number of sex partners in the past three years was not a contributing factor, similar to the finding of Lagarde *et al.*,¹⁹ since very few women reported having more than one sex partner in the past three years. Finally, following the experience of sexual violence, women often become more conservative in their sexual behavior.¹⁸

Although substance use was significant when the variables were entered into the regression as a block, no individual variable within this block attained individual significance, probably because they were relatively infrequent within the sample. Cultural practices such as vaginal drying herbs and scarification have been reported to be associated with HIV status in other studies,^{20,21} but those results did not replicate when a larger array of explanatory variables were also assessed in this sample.

Even though other studies have found a significant interaction of other STDs with HIV acquisition or transmission²¹ such findings did not replicate with this sample of women. Only syphilis and HHV-8 were laboratory confirmed due to budget constraints and information about the other STDs relied on women's self-report. For many STDs, this likely resulted in substantial under-reporting. If it had been possible to utilize biological measures for all of the STDs, the results may have been quite different. Given overwhelming evidence that genital ulcer disease is associated with HIV acquisition and transmission²³ of and the absence of laboratory evidence in our data, it is probable that these diseases were under-reported. However, even for syphilis, which was laboratory confirmed, there was no significant relationship to HIV in this sample of women. One possible explanation suggested by Rottingen *et al.*²² may be that publication bias simply favors overestimation of STDs as HIV transmission facilitators. Other reasons are the possibility that women either did not recall or did not disclose stigmatizing conditions and the possibility that the variance associated with STDs overlapped with another measure. Clearly, when assessed in the presence of all other potential contributors, the relationship of STDs as measured here to HIV infection was minimal. In future research, the use of biomedical measures rather than self-report STD data is strongly recommended.

Finally, the interview data for this study was collected by a face-to-face interview, a methodology that may have suppressed reporting sensitive behaviors such as anal intercourse. Brody and Potterat²⁴ speculated that under-reporting of sexual behaviors other than penile-vaginal intercourse is widespread and that sexual practices such as anal intercourse are more prevalent in Africa than has traditionally been reported. Audio computer assisted

self-interviews (A-CASI) have sometimes been found to yield higher reports of sensitive behaviors, but this study utilized individual interviews. However, an unrelated study in neighboring Zimbabwe found no significant reporting differences between A-CASI and face-to-face interviews, a finding that calls into question whether this was a substantial problem for this sample.²⁵

In summary, when the current discussion about whether medical injections could improve our explanations of HIV acquisition in sub-Saharan Africa was assessed using an existing data set that also measured most other variables already known to have an association with HIV prevalence, medical injections emerged as a strong predictor. Reliance on disposable needles increasingly has become standard practice, certainly at UTH where these data were collected. Future research would, hopefully, find lower associations of HIV with medical injections since we would expect to see a decrease in iatrogenic transmission as use of disposable syringes increased. However, future prospective research that also gathers reasons for the medical injections, information about the health care settings in which they were administered, assesses the injection apparatus after use, includes measurement of who administers the injection, includes measures of other 'punctures' such as for dental work or scarification, includes a larger array of biomedical STD test results, and collects parallel data from both women and their primary partners is recommended.

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