

Research On Human Immunodeficiency Virus (HIV) In Malawi: The Johns Hopkins University- Ministry of Health (JHU-MOH) Project

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BACKGROUND

The situation in Africa

The prevalence of HIV infection shows considerable variability within Africa among high risk urban groups (mainly prostitutes and sexually transmitted disease clinic patients). It is over 35 % in Rwanda, Uganda, Zambia, Kenya, Malawi, Tanzania and Zaire¹, 10-35 % in most west and central African countries, 2.5-10 % in South Africa, Mozambique, Cameroon and Benin, 0.3-2.5 % in Botswana and Senegal, and less than 0.3 % in Somalia and the north African countries^{1,2}. HIV infection among low risk urban groups is much less common and the highest HIV seroprevalence (more than 10 %) has been reported from Uganda and Zambia².

Heterosexual and perinatal transmission are the main routes of spread of HIV in Sub-Saharan Africa. Transmission of the virus through breast milk has recently been investigated and tentatively quantified^{3,4}. This mode of transmission has the potential for considerable policy implications regarding infant feeding and child health in developing societies. It has been estimated that by the early 1990s about 2.5 million women of childbearing age in Africa were infected with the virus; these women gave birth to about 2 million infants of whom about 500,000 infants (25 %) were infected with HIV⁵. These figures highlight the challenge posed by HIV on the health, social, and economic development and the demographic structure of Sub-Saharan Africa.

The JHU-MOH Research Project

The current research project commenced in 1989 and is a collaborative effort between the Johns Hopkins University School of Hygiene and Public Health in Baltimore, U.S.A. and the Malawi Ministry of Health. The project, which included studies on different aspects of HIV infection, was approved by scientific and ethical committees in Malawi and in the U.S.A. A resident research team at Queen Elizabeth Central Hospital (QECH) in Blantyre conducts the different activities of the project and acts as a liaison between scientists in the country and abroad.

The purpose of this paper is to summarize briefly the studies completed by the JHU-MOH project to date and to draw conclusions and recommendations from these investigations. Further details on these studies can be found in papers by the JHU-MOH project cited throughout the text.

MATERIALS AND METHODS

The study design

At the outset, a small cross-sectional study was conducted to determine HIV-1 seroprevalence among women attending prenatal visits at QECH in Blantyre and Kamuzu Central Hospital (KCH) in Lilongwe, the main referral hospitals in the country. This was followed by a longitudinal study to determine risk factors for HIV-1 infection among pregnant women attending antenatal visits at QECH. Women from this study were recruited at the time of delivery to a prospective study to determine risk factors of HIV transmission from infected mothers to their infants and on uninfected women to determine biologic and behavioral co-factors of HIV seroconversion. Seroconversion is defined as change of HIV serology tests from negative to positive (a seroconverter is a person who displays that change).

Data collection and physical examination

After obtaining informed consent, socio-demographic information and medical, reproductive and pregnancy history were obtained. The interviews were conducted by trained study workers during the first antenatal visit, after delivery and at subsequent postnatal visits. Home tracing of women lost to follow-up was done when necessary. At each visit thorough physical and speculum aided pelvic examinations were conducted by trained midwives and appropriate specimens were collected.

Specimen collection

Vaginal fluid, cervical swabs, Pap smear and cervico-vaginal lavage for human papilloma virus (HPV) were routinely collected at regular intervals. Blood was collected for HIV testing, syphilis serology, white blood count and differential, and lymphocyte immunophenotyping. Breast milk specimens, and, on a limited number of women, placental sections were collected. Colposcopic examination was conducted by a gynaecologist on women who had an abnormal Pap smear or a positive HPV result. From infants, blood was collected at age 12 and 18 months for HIV-1 testing, and malaria smears were routinely prepared in the follow-up visits.

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Laboratory analyses

A group of trained technicians conducted serum analysis using ELISA test (Wellcozyme, Wellcome Diagnostics) for the detection of antibody to HIV-1. The procedures followed were regularly assessed for quality control and assurance. All specimens were initially screened by ELISA and those testing positive for HIV-1 were confirmed by Western blot (Bio-Rad Laboratories). Syphilis serology was performed using the rapid plasma reagin (RPR) test for screening and fluorescent treponemal antibody test (FTA) for confirmation. Microscopic examinations were done on vaginal wet mounts to detect *T. vaginalis*, yeast forms, clue cells and white blood cells. Cervical swabs for *N. gonorrhoea* (GC) were inoculated into culture media prepared in the laboratory in Blantyre and placed in candle extinction jars. The jars were incubated at 36°C for 24-48 hours and isolates were presumptively identified by colony and Gram stain morphology and by oxidase reaction. Dacron cervical swabs for chlamydial fluorescent antibody test (Microtrak Direct Specimen Test) were applied to the slide, fixed with methanol, and frozen at 20°C for later processing in the United States. HPV detection was done by polymerase chain reaction (PCR) on cervicovaginal cells obtained by lavage. Samples were shipped to the Johns Hopkins University in the U.S. where analysis was done. WBC counts were followed by lymphocyte immunophenotyping using fluorescent monoclonal antibodies (Becton Dickinson). Flow cytometric analysis of CD4 and CD8 lymphocytes and white blood cell differentials were done with a FACScan (Becton Dickinson) available at the JHU laboratory in QECH.

Data management and analysis

Data collected were systematically checked for completeness and entered onto micro-computers in Blantyre. After further data management, univariate and multivariate analyses were conducted as appropriate. These included descriptive statistics, rates of morbidity and mortality, measures of associations, and confidence intervals. Survival analysis was used to determine rates of events, and multiple logistic regression was used for adjustment of potential confounders, appraisal of interactions and assessment of major predictors of outcome.

RESULTS AND DISCUSSION

Prevalence/Incidence of HIV-1

Of 6605 women who were interviewed and tested at QECH between October 1989 and October 1990, 1505 (22.8%) were HIV-1 seropositive. For comparison, the seroprevalence of HIV-1 among pregnant women attending antenatal clinics at QECH in Blantyre for the period 1985-1993 and at KCH for the period 1987-1990 is shown in Table 1. There is a clear trend of increase in prevalence over time. These estimates are cross-sectional in nature and should not be extrapolated without caution to other urban or rural communities of Malawi.

Table 1. Seroprevalence of HIV-1 among pregnant women attending antenatal clinics in QECH and KCH, 1985-1993.

Month/Year	QECH		KCH	
	# tested	Prevalence %	# tested	Prevalence %
Jun. 1985	200	2.0	—	—
Jun. 1987	85	8.2	184	8.2
Dec. 1988	247	18.6	214	16.4
Oct. 1990	845	21.9	201	17.9
Oct. 1991	404	25.9	—	—
Jul. 1992	291	27.2	—	—
Jan. 1993	437	31.6	—	—

The point estimates of the incidence of HIV-1 seroconversion at QECH and KCH for the period 1987 to 1989 was estimated as 10% and 7.5%, respectively⁶. The rate of HIV-1 seroconversion after delivery among a cohort of 694 HIV-1 seronegative mothers who were enrolled at delivery is shown in Table 2. The rate of HIV-1 seroconversion increases over time suggesting that women in Malawi are at increased risk during the postnatal period. The lower rates in the first year could be due to the traditional practice of sexual abstinence in the first six months after delivery in this community⁷.

Table 2. Rate of HIV-1 seroconversion after delivery, QECH, Blantyre 1989-1992.

Months	HIV-1 Sero-Converters	Person-Semester*	Rate (%)
0-6	5	559.4	0.90
7-12	9	473.5	1.90
13-18	10	406.8	2.46
19-24	11	253.5	4.34

* Number of persons at risk of HIV-1 seroconversion X Number of six-month periods (semester) in which these persons are at risk. All persons were considered at risk of HIV-1 seroconversion until the time they seroconverted.

Rates of sexually transmitted diseases (STDs)

The incidence of gonorrhoea, trichomoniasis, genital ulcers and genital warts among the cohort of 644 HIV-1 seropositive and 677 HIV-1 seronegative women is shown in Table 3. The cumulative incidence of these diseases was significantly higher in HIV-1 seropositive than in HIV-1 seronegative women. The higher rate of new STDs in HIV-1 infected women may represent increased susceptibility, increased exposure or, in the case of genital warts, compromised immune status of the woman. The consistent relationship between STDs and HIV-1 emphasizes the need for a coordinated intervention approach to optimize management and control of STDs⁸.

Table 3. Incidence of sexually transmitted diseases among HIV-1 seropositive and seronegative women, QECH, Blantyre November 1989 to November 1992.

STD	HIV-1 positive %	HIV-1 negative %	P-value
Gonorrhoea	19.8	7.9	<0.001
Trichomoniasis	51.3	35.6	0.009
Genital ulcer	26.9	9.3	<0.001
Genital warts	23.6	13.6	0.030

Risk factors associated with HIV-1 seropositivity and seroconversion

Sexually transmitted diseases. In the cross-sectional study, genital warts were found in 124/1479 (8 %) HIV-1 seropositive vs 110/5031 (2 %) HIV-1 seronegative women (odds ratio=4.1). Genital ulcer disease was present in 166/1479 (11 %) HIV-1 seropositive vs 277/5031 (5 %) HIV-1 seronegative women (odds ratio=2.2). Cervical ectopy was not associated with HIV seropositivity (ectopy was found in 31 % of HIV-1 infected vs 28 % of HIV-1 uninfected women). Clinical indicators of cervicitis (abnormal cervical discharge and cervical bleeding) were more common in HIV-1 infected women, regardless of the presence of cervical infection, making these clinical indicators less useful in STDs evaluation of HIV-1 infected women⁹.

High socio-economic status and STDs. Women of higher socio-economic status (SES) and women with current STDs were both more than twice as likely to be HIV-1 infected. Higher SES was defined as husband's education of more than eight years. HIV-1 seroprevalence increased with SES, from 26 % to 42 % in women with diagnosed STDs and from 11 % to 22 % in women without STD diagnosis. The presence of diagnosed STDs approximately doubled the risk of having HIV-1 infection irrespective of SES: 22 % to 42 % among high SES women and 11 % to 26 % among low SES women. STDs were positively associated with HIV-1 infection (OR=2.73), but not with higher SES. These results suggest that women and their husbands/partners of higher SES adopt more high risk behaviours which predispose to HIV-1 infection. Such behaviours are apparently encouraged by their economic ability to seek medical care and treatment for bacterial STDs¹⁰. The findings have policy implications related to lack of adequate knowledge on transmissions of HIV-1 even among the "elite" groups of higher SES. Therefore intervention strategies to control STDs should include all segments of the population.

Vaginal agents. Use of traditional vaginal agents (desiccants, leaves, stones, etc.) for treatment of itching and vaginal discharge or for tightening purposes were investigated as risk factors for HIV-1 infection because these practices have been reported to be associated with HIV-1 infection in other African countries^{11,12}. Forty five percent of women attending the antenatal clinic at QECH reported self-treatment of vaginal discharge and itching and 13 % (886) reported using vaginal agents for tightening of the vaginal wall. Of HIV-1 infected women 17 % used intra-vaginal agents for treatment in comparison to 14 % of uninfected women (OR=1.2; p=0.01). Although the relative risk is small, the risk attributable to these agents could be substantial due to their fairly common use in the population studied¹³.

Human papilloma virus (HPV) infection and cervical lesions in HIV-1 infected women. Samples on 286 postpartum women were examined to document the association between HPV infection, squamous intra-epithelial lesion (SIL) and HIV-1 infection. Cervical SIL was detected in 13 % of HIV-1 seropositive vs 6 % HIV-1 seronegative women. SIL increased with decreasing CD4 cells among HIV-1 infected women (p trend=0.06). HPV was detected in 20 % of the HIV-1 seronegative, but in as high as 38 % of HIV-1 seropositive women with less than

300 CD4 cells. Among women followed longitudinally, cervicovaginal lavage specimens were collected on 104 HIV-1 seropositive and seronegative women at two time points approximately one year apart. Seropositive women were more likely than seronegative women to have HPV newly detected and to show persistent HPV in the follow-up samples (Table 4). Genital neoplasms may become more frequent in women of high HIV prevalence areas. Therefore, closer gynaecologic screening of high risk women in these areas is warranted as cervical cancer is becoming a major cause of mortality in Africa (Chiphangwi et al, unpublished data).

Table 4. Human Papilloma Virus (HPV) expression among HIV-1 seropositive and HIV-1 seronegative women in two follow-up visits, QECH 1992.

HPV status From	To	HIV-1 Sero-positive	HIV-1 Sero-negative	P-Value
HPV(-)	HPV(+)	16/30 (53%)	13/48 (27%)	0.02
HPV(+)	HPV(-)	3/13 (23%)	9/13 (69%)	0.02
HPV(+)	HPV(+)	10/13 (77%)	4/13 (31%)	0.02
HPV(-)	HPV(-)	14/30 (47%)	35/48 (73%)	0.02

Maternal HIV-1 p-24 antigen and vertical transmission.

Using a quantitative p-24 antigen ELISA test, 123 serum specimens from third trimester HIV-1 serostatus known women were tested to determine whether the level of maternal p-24 antigenemia was correlated with HIV-1 transmission to their babies. These 123 women included 54 HIV-1 seropositive women with HIV-1 infected babies, 49 HIV-1 seropositive women with HIV-1 uninfected babies, and 20 HIV-1 negative women as controls. The HIV-1 serostatus of the baby was established at 12 months and confirmed at 18 months of age. Eighteen of 26 HIV-1 seropositive mothers (69 %) with detectable p-24 antigen had infected infants, whereas 36 of 77 HIV-1 seropositive mothers (47 %) without detectable p-24 antigen had infected infants (OR=2.6, p=0.04). These findings suggest that transmission of the virus to the fetus occurs either very early after infection or late in the course of the disease i.e., when there are not enough antibodies to the HIV-1 p-24 antigen.

Infant mortality and spontaneous abortion. A history of childhood mortality in the previous pregnancy was present in 35 % HIV-1 seropositive vs 15 % HIV-1 seronegative mothers. Infant mortality and under three year mortality was 77 per 1000 and 119 per 1000, respectively, in seronegative mothers. For seropositive mothers, however, mortality rates were higher; 171 per 1000 infant mortality and 292 per 1000 under three year mortality. Spontaneous abortion was reported more often by HIV-1 seropositive than seronegative mothers (15 % vs 7 %), and was associated with history of previous abortion, history of STDs, confirmed syphilis serology and young maternal age¹⁴.

The mortality experience and associated factors during the first year were studied on the babies of 691 HIV-1 infected and 687 uninfected mothers in the cohort study. Infant mortality was significantly higher for babies of HIV-1 seropositive mothers than for babies of HIV-1 seronegative mothers (Table 5). Short gestational age was associated with lower survival probabilities and occurred significantly more often in infants of HIV-1 seropositive

mothers (13.9 %) than in infants of seronegative mothers (5.4 %). Pneumonia, fever, failure to thrive and diarrhoea were the most common proximate causes of death besides prematurity/low birth weight in babies of seropositive mothers. Excess mortality among infants aged 3-12 months of seropositive mothers could be attributed to HIV-1 infection¹⁵.

Table 5. Survival probabilities (and Standard errors) for infants of HIV-1 seropositive and HIV-1 seronegative mothers, QECH Blantyre, 1992.

Month	Maternal HIV-1 Status	
	Seronegative	Seropositive
3	0.979 (0.006)	0.952 (0.008)
6	0.970 (0.007)	0.889 (0.013)
9	0.956 (0.008)	0.637 (0.015)
12	0.944 (0.010)	0.802 (0.017)

In a related analysis, of 367 babies born to HIV-1 seropositive mothers who were alive and tested for HIV-1 antibody at 12 months or after, 84 (22.9 %) were positive by ELISA and Western blot testing. Combining this information with the probability of death before 12 months for babies born to seropositive and seronegative mothers (0.198 and 0.056, respectively), the mother-to-infant HIV transmission rate was estimated as 34.5 %. In this population, all babies who were HIV-1 seropositive at 12 months (N=51) were also HIV-1 seropositive at 18 months or later, suggesting that loss of maternal antibodies to HIV-1 occurs by age 12 months.

Risk factors for incident HIV infection. The risk factors with the strongest associations with HIV-1 seroconversion among women who were previously seronegative were trichomoniasis (OR=5.75, 95 % C.I 1.11-12.70), sexual intercourse with husband/partner using condom (OR=6.50, 95 % C.I 1.28-31.90), and maternal age below 25 years (OR=2.00, 95 % C.I 1.00-4.06). While the biological mechanism connecting trichomoniasis with HIV infection is not clear, it is possible that this is a surrogate for other diagnosed STDs or high risk sexual activity in general. The finding that condom usage is a risk factor for seroconversion runs contrary to conventional understanding of the role of condoms in preventing HIV infection. A likely explanation is that women did not use condoms consistently or condom use is only a marker of high risk behaviours. Data on incident HIV-1 infection in specific populations are critical to monitor epidemic trends, identify modifiable risk factors and plan interventions such as vaccine clinical trials⁷.

Knowledge, attitudes, beliefs and practices (KABP). Based on STDs incidence and reported sexual history, HIV-1 seropositive women appeared more likely than HIV-1 seronegative women to over-report consistent condom use. Similarly, both seropositive and seronegative women under-reported sexual activity. These findings suggest that reported condom use is less reliable in seropositive than in seronegative women. Reported condom use and sexual history, variables commonly used in program evaluation, should be validated with biologic markers¹⁶.

To evaluate knowledge and attitudes over time 688 HIV-1 seropositive and 690 HIV-1 seronegative women

were counselled and educated on several issues regarding coping with effects of AIDS, condom use and means of minimizing transmission. There was improvement in knowledge over time but some problems regarding routes of transmission were apparent especially among young and low socio-economic status mothers. Religion, high cost and lack of sexual pleasure were not the main barriers to condom usage. Appropriate education and counselling has a positive impact on knowledge of HIV/AIDS and should be carried out as often as possible, and antenatal care clinics provide a good opportunity¹⁷.

CONCLUSIONS

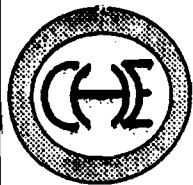
The studies conducted on pregnant women in Blantyre show that HIV-1 seroprevalence has been rising over time. The rate of transmission from the mother to the infant is high in this population. HIV-1 infection of the mother substantially contributes to fetal wastage and to the high levels of infant and childhood mortality in Malawi. HIV-1 seropositive mothers are more susceptible to other STDs and show immunological derangements similar to that of women from developed countries¹⁸. The findings justify the health and socio-demographic concerns if the current situation prevails. Several factors with significant policy implications have been identified and could be considered in intervention programs to reduce transmission of the virus in the country. Although our investigation, by design, excludes male partners who also contribute to this epidemic, their role in sustaining the epidemic should not be underestimated. Therefore effective intervention activities should have no gender restrictions. Prevention of STDs, including adequate clinical services for diagnosis and treatment, is a priority identified by the WHO Global Program on AIDS for many countries of Sub-Saharan Africa and confirmed by these studies in Malawi. All segments and economic strata of the population should be targeted by educational messages. For pregnant women, the antenatal clinic is a reasonable setting but has obvious limitations. In a situation where the prevalence of HIV-1 is high, it would be appropriate to disseminate the information to adolescent girls and nonpregnant women. The rate of new HIV-1 infection increases after delivery and is associated with modifiable behaviours, making the postpartum period an appropriate time to consider for STD control, educational programs and HIV/AIDS vaccine trials. In this direction, the JHU-MOH project has begun an extended study on preparation for AIDS/HIV vaccine evaluation (PAVE) among pregnant women. Until a globally firm intervention strategy is reached, conventional measures such as appropriate counselling, education, diagnosis and treatment of STDs, and promotion of condom use are recommended.

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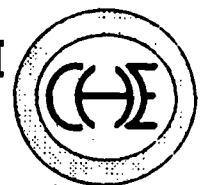
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