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HIV-1 Seroprevalence among Pregnant Women in Rural Uganda: A Longitudinal Study over Fifteen Years

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Key Words
HIV infection · Pregnant women · Sentinel surveillance · Uganda · Syphilis

Abstract
Introduction: In order to determine the development of the prevalence of HIV infection in rural Western Uganda, data of epidemiological studies conducted in 2001 and 2007 were compared to study data from 1993. ***Methods:*** In 2001 (n = 466) and in 2007 (n = 486), one group each of clinically healthy pregnant women of a local prenatal care department were enrolled in the study and anonymously screened for HIV-1. For both groups, informed consent was obtained prior to enrolment. Testing for HIV was done by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot. In addition, age and antibodies against syphilis were determined as risk factors of HIV infection. ***Results:*** The seroprevalence of HIV-1 infection did not decrease significantly over this time period, dropping from 28.3 to 25.1% between 2001 and 2007, but the prevalence of syphilis antibodies decreased from 27.9 to 11.1%. The data of 2001 and 2007 were compared to a third cohort from 1993, in which 21.5% of pregnant women were HIV-1-positive and 31.1% were Treponema pallidum hemagglutination assay (TPHA)-positive. ***Conclusion:*** The current prevalence of HIV-1 infection in Uganda is still high and there is a need for further promotion of HIV prevention and control services.

Introduction

Over 25 years ago, the human immunodeficiency virus, HIV-1, was discovered. Up till now, no protective vaccine has been developed. Particularly in sub-Saharan Africa, HIV/AIDS is one of the leading causes of death among young adults. Prevalence data are vital for tracking the progress of the HIV epidemic. In most countries in sub-Saharan Africa, this is monitored by sentinel surveillance among pregnant women attending an antenatal clinic (ANC). Because heterosexual intercourse is the main mode of HIV transmission in these countries, the prevalence of infection among attendees of ANCs is usually assumed to be representative of the general population (males and females of reproductive age [1, 2]). A trend in HIV-1 prevalence in a particular region cannot be determined by an anonymous sentinel surveillance among pregnant women attending an ANC at one time. Therefore, longitudinal surveillance needs to be performed over several years. The present national HIV-1 sentinel surveillance system currently involves 19 ANCs located in 18 of the country's 56 districts. Furthermore, the national PMTCT (Prevention of Mother-To-Child Transmission) program, which was created by the Ugandan Ministry of Health in 2000, currently involves health facilities in 35 districts.

This longitudinal epidemiological study was conducted to examine the prevalence of HIV-1 infection in cor-

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relation to other sexually transmitted diseases (STDs) and age ranges in rural Uganda over a period of almost 15 years.

Material and Methods

Integrated Population and Sample Collection

At three time points, each about 6–8 years apart, pregnant women attending a dispensary in South Western Uganda were examined for HIV antibodies: there were 409 in 1993, 466 in 2001 and 486 in 2007. Our data were assessed as an 'Anonymous and Unlinked Sentinel Sero-Surveillance Study' in a small unlisted ANC unit in Western Uganda, in addition to the 19 mentioned ANCs of the country's national HIV-1 sentinel surveillance system. Bujumbura Health Center is provided by the Catholic Church and is mainly supported by the Banyatereza Sisters. Located south-east of Hoima town, it is frequented by urban as well as rural patients from different parts of the country district. After having given informed consent, female attendees of an antenatal clinic who were up to 38 weeks' pregnant and comparable with regard to their age (11–54 years old) were enrolled in this study as representatives of the fertile, sexually active population. Study samples were obtained anonymously and consecutively at each visit within a period of 6 months. All the women included came from the local district and were ethnically Bunyoro. Study participants were also screened for syphilis antibodies in correlation to their HIV status. Immediately after data analysis, our results were reported to the Ugandan Ministry of Health as a cooperating partner.

Serology

The sera samples for HIV and syphilis were obtained anonymously from pregnant women. First testing for HIV antibodies was done by ELISA (New ABBOTT AxSYM HIV Ag/Ab Combo Assay, ABBOTT Wiesbaden, Germany). Nonvalid, unclear and reactive samples were confirmed by Western blot (INNO-LIA^{T-MM} HIV I/II Score, Innogenetics, STD Technology for Health Care, Hannover, Germany). In 2007, confirming tests (Western blot) showed unspecified results in six blood samples (1.2%), which would have required a further blood sample. Because our 'Anonymous and Unlinked Sentinel Sero-Surveillance Study' investigated one 'status quo' at one time, these unspecific samples were excluded.

Syphilis serology was done by Treponema pallidum hemagglutination assay (TPHA, Mast Diagnostica, 'Laboratoriumspräparate GmbH', Reinfeld, Germany).

Statistical Analysis

Data management and statistical analysis were conducted in SPSS version 15.0 (SPSS Inc., Chicago, Ill., USA). Continuous variables were summarized by means of the median, the interquartile (IQR, 25th–75th percentile), and the minimum to maximum range. For categorical data, frequency counts and percentages were calculated. Between-group comparisons were assessed with the Mann-Whitney-U test for continuous variables and the Pearson χ^2 test for categorical variables. All tests were done at the 5% two-sided significance level. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated and considered statistically significant if the CI exceeded 1.0. No adjustment for multiple testing was conducted. We calculated HIV-1 prevalence and exact binomial CI. The OR obtained by running logistical regression models were used to describe changes at the two time points regarding the risk of HIV-1 infection. The χ^2 for linear trend tests was used for univariate analysis.

Table 1. Age distribution of total number of participating pregnant women in 2001 and 2007

Year	2001		2007	
	n	%	n	%
Total n	466		486	
Age, years				
≤15	6	1.3	1	0.2
15–19	89	19.1	103	21.2
20–24	110	23.6	155	31.9
25–29	114	24.5	109	22.4
30–34	68	14.6	57	11.7
35–39	55	11.8	31	6.4
40–44	21	4.5	5	1.0
>44	2	0.4	3	0.6
Unknown	1	0.2	22	4.5
Median, IQR	26	20–31	24	20–28

Median and IQR were determined. Eight age-ranges were defined (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44 and >44 years old). In 2001, only 1 (0.2%) and in 2007, 22 (4.5%) pregnant women were not aware of their age.

Results

Demographic Data

A total of 952 women met the inclusion criteria and were available for analysis, 466 (48.9%) in 2001 and 486 (51.1%) in 2007.

The median age of the entire study population was 25 years (range 11–54 years). The women in the 2007 study group were significantly (p = 0.001) younger than the women in the study group of 2001: the median age of the study population in 2001 was 26.3 years ranging from 11 to 51 years, whereas in 2007 the median age was 24.7 years ranging from 14 to 55 years. So, in 2001, most participants belonged to the age group 25–29 years and in 2007, the age peak was the period 20–24 years (table 1).

Altogether, the population surveys did not differ significantly (p = 0.235). No differences could be found

Table 2. HIV-1 serology status by age group

Year		2001			2007	
		n	%		n	%
Patients, total n		466			486	
Age, years						
<15	positive	2	33.3	positive	0	0.0
	negative	4	66.7	negative	1	100.0
15–19	positive	24	27.0	positive	19	18.4
	negative	65	73.0	negative	83	80.6
				invalid	1	1.0
20–24	positive	27	24.5	positive	41	26.5
	negative	83	75.5	negative	111	71.6
				invalid	3	1.9
25–29	positive	42	36.8	positive	28	25.7
	negative	72	63.2	negative	81	74.3
30–34	positive	16	23.5	positive	15	26.3
	negative	52	76.5	negative	41	71.9
				invalid	1	1.8
35–39	positive	14	25.5	positive	8	25.8
	negative	41	74.5	negative	22	71.0
				invalid	1	3.2
40–44	positive	6	28.6	positive	1	20.0
	negative	15	71.4	negative	4	80.0
>44	positive	1	50.0	positive	0	0.0
	negative	1	50.0	negative	3	100.0
Unknown	positive	0	0.0	positive	10	45.5
	negative	1	100.0	negative	12	54.5
Median:	26, IQR: 20–31			24, IQR: 20–28		
Summarized	positive	132	28.3	positive	122	25.1
	negative	334	71.7	negative	358	73.7
				invalid	6	1.2

Number and age distribution of HIV-1-positive and -HIV-negative pregnant women in the 2 studies in 2001 and 2007.

in the type of residence: all 2001 and 2007 participants belonged to rural populations.

With regard to the validity of data on age, 456 (99.8%) of the women in 2001 and 464 (95.5%) in 2007 knew their age i.e. one patient in 2001 and 22 in 2007, respectively, did mention that they were not aware of their exact age (table 1). All other women and/or girls gave their age with or without a passport or birth certification.

Prevalence of HIV among Pregnant Women

In 2001, 132 (28.3%) of the 466 pregnant women without symptoms were found HIV-positive by confirmed testing (Western blot) and 334 (71.7%) were HIV-negative.

In comparison, in 2007, 122 (25.1%) of the 486 participating pregnant women tested HIV-positive and 358 (73.7%) were HIV-negative which shows a decrease in HIV prevalence of 3.2%, which is not significant ($p = 0.129$).

In 2001, the highest percentage of HIV positivity was found among pregnant women in the age group 25–29 years. In 2007, this was a younger age group of 20–24 years, i.e. the peak of HIV prevalence in pregnant women changed (table 2).

Seroprevalence of Syphilis Antibody Positivity among Pregnant Women

In 2001, 130 out of 466 pregnant women tested Treponema pallidum hemagglutination assay (TPHA)-positive (27.9%). In 2007, only 54 (11.1%) out of 488 pregnant women were syphilis antibody-positive, i.e. there was a significant ($p < 0.001$) reduction of seroprevalence of TPHA positivity. In 2001, the highest prevalence of 32.7% was found in the age group of 20–24 years. A second peak was found in the age group of 40–44 years with 33.3%. In 2007, TPHA positivity, in correlation to age, peaked twice: first among the 35- to 39-year olds with 29.0%, and secondly, among the older than 44-year-olds with 66.7%. This last value cannot be considered striking because the second peak was caused by 2 pregnant women out of only 3 TPHA-positive patients, i.e. the number of random samples was very low (table 3).

Simultaneous HIV and Syphilis Infection

An interesting correlation emerged between simultaneous HIV and syphilis infection. In 2001, 45 out of 466 pregnant women (9.7%) were simultaneously infected with HIV-1 and syphilis. Thus, out of 132 pregnant women who were seroreactive for HIV-1, and out of 130 pregnant women who were positive for syphilis antibody in 2001, 45 women had acquired both infections. In 2007, this infection rate decreased to only 3.9%, i.e. 19 out of 122 HIV-positive women and 54 TPHA-positive women were HIV-positive and TPHA/syphilis antibody-positive, which represents a considerably significant reduction in simultaneous HIV and syphilis infection (OR = 2.63, 95% CI 1.51–4.56, $p < 0.001$) (table 4; fig. 1).

Discussion

The prevalence of HIV-1 infection among women of reproductive age was and is increasing worldwide [3–5]. Uganda is a country known to make a difference.

Table 3. Syphilis serology status by age group

Year		2001			2007	
		n	%		n	%
Total n		466			486	
Age, years						
<15	positive	1	16.7	positive	0	0.0
	negative	5	83.3	negative	1	100.0
15–19	positive	22	24.7	positive	3	2.9
	negative	67	75.3	negative	98	95.1
				invalid	2	1.9
20–24	positive	36	32.7	positive	15	9.7
	negative	74	67.3	negative	137	88.4
				invalid	3	1.9
25–29	positive	33	28.9	positive	9	8.3
	negative	81	71.1	negative	97	89.0
				invalid	3	2.8
30–34	positive	17	25.0	positive	10	17.5
	negative	51	75	negative	46	80.7
				invalid	1	1.8
35–39	positive	14	25.5	positive	9	29.0
	negative	41	74.5	negative	21	67.7
				invalid	1	3.2
40–44	positive	7	33.3	positive	1	20.0
	negative	14	66.7	negative	4	80.0
>44	positive	0	0.0	positive	2	66.7
	negative	2	100.0	negative	1	33.3
Unknown	positive	0	0.0	positive	5	22.7
	negative	1	100.0	negative	97	72.7
				invalid	3	4.5
Median	26, IQR: 20–31			24, IQR: 20–28		
Summarized	positive	130	27.9	positive	54	11.1
	negative	336	72.1	negative	419	86.2
				invalid	13	2.7

Number and age distribution of syphilis-positive and syphilis-negative pregnant women in the 2 studies in 2001 and 2007.

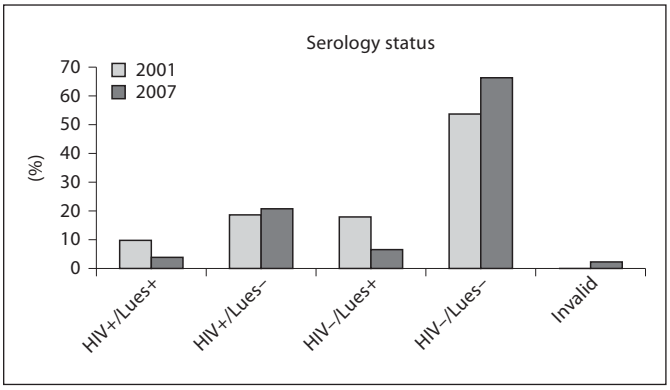

Fig. 1. Data of table 4 displayed in a column diagram.

Table 4. Simultaneous HIV-1 and syphilis seroprevalence in 2001 and 2007

Year		Frequency	Percentage
2001	HIV+ and syphilis+	45	9.7
	HIV+ and syphilis–	87	18.7
	HIV– and syphilis+	84	18.0
	HIV– and syphilis–	250	53.6
	Total	466	100.0
2007	HIV+ and syphilis+	19	3.9
	HIV+ and syphilis–	101	20.8
	HIV– and syphilis+	33	6.8
	HIV– and syphilis–	322	66.3
	Invalid	11	2.3
	Total	486	100.0

Reduction of HIV-1 and syphilis double infections from 2001 to 2007 (highly significant $p < 0.0001$). Positive: + and negative: –.

Childbearing women are a well-identified group who represent the young, sexually active population. The prevalence of HIV among pregnant women attending ANCs included in the national sentinel surveillance program has significantly declined in the last years [6]. In Ugandan urban areas, between 90 and 92% of pregnant women attend an ANC at some time during their pregnancy. However, a routine surveillance system such as that in Uganda often shows weaknesses such as missing data, inconsistencies in the samples and a lack of precision in estimates of HIV seroprevalence. The findings cannot be generalized throughout the country [7, 8].

Our objective, therefore, was to continue a sentinel surveillance which was started by our group in the mid-1990s, to determine seroprevalence trends among pregnant women in rural Western Uganda over a sufficient period of time, i.e. about 15 years. In this study, at two time-points, 6 years apart, i.e. in 2001 and 2007, the prevalence of HIV among pregnant women attending an ANC was determined and the results were compared. The data were collected as a continuation of a study that had already taken place more than 7 years before; in the 1990s, we had undertaken the first study in the same Ugandan region [9] that showed an overall HIV seroprevalence among pregnant women of 21.5% (88 out of

409). Altogether, we could now sample the HIV serology of 1,361 pregnant women participating in 3 serosurveys in total.

In 1993, 11.5% of pregnant women were infected with both HIV and syphilis. The overall syphilis seroreactivity was 39.1% (160 out of 409 women) and was linked significantly to HIV infection ($p < 0.0005$) [9]. This emphasizes the fact that the presence of genital ulcers or similar lesions increases the susceptibility of HIV infection as reported in many previous studies [10, 11]. The pathology which takes place is the attraction and active infiltration of lymphocytes and macrophages in these lesions and ulcers which facilitate and even force the entrance of the HIV into the blood circulation [12]. Patients with any STD in the past 5 years – as reported in another study [13] – had a significantly higher probability to be infected with HIV than those without such a disease history (26%; OR 2.08).

Our main finding was that the HIV-1 seroprevalence rate among pregnant women attending an ANC in rural Uganda in 2001 amounted to 28.3%. This percentage represents a considerable increase (6.8%) in 8 years when compared to the rate of 21.5% in 1993 among pregnant women in the same area [9].

In 2001, HIV positivity peaked among pregnant women in the age group 25–29 years, and in 2007 in the younger age group of 20–24 years. There was shift of peak HIV prevalence to younger pregnant women. The comparison does reflect, however, the whole pregnant population participating in the two anonymous sentinel surveillances of the years 2001 and 2007. Therefore, our studies in Uganda in sub-Saharan East Africa may not necessarily reveal a real shift of HIV infection to an earlier onset in pregnant women.

This high prevalence of HIV-1 infection, particularly among young fertile women, is similar to the results of other East African population-based studies [14]. The increasing incidence of HIV in rural areas is often concentrated in younger age groups. The increased morbidity and mortality rates and the fertility problems associated with HIV in the late 1990s may reflect the still-existing lack of behavioral change or improvement in STD case management [15]. These findings indicate that, in spite of the high level of HIV awareness in Uganda, the seroprevalence rate still rose in rural areas in the late 1990s, which correlates with data from other studies [16]. An inversion of this situation may be expected in the next few years, as this trend was seen in these latest data of 2007 when we found a reduction of HIV seropositivity to 25.1%. Our study showed an overall reduction of HIV prevalence of 3.2% in rural Uganda from 2001 to 2007. Over the past 20 years, several other studies have also shown a considerable decline of HIV prevalence among pregnant women attending ANCs in urban areas. For years, urban data have shown a decrease of HIV seroprevalence in the population [8]. This trend is less pronounced in the countryside. Even if our decrease in HIV seroprevalence seems to be minor in comparison to other studies reporting higher reduction rates of HIV infection [16], we did uncover an enormous decrease in syphilis seroprevalence: from 27.9 to 11.1%. Thus, this decrease indicates a tremendous behavioral change in the population, and a continuation of this change may prospectively further reduce seroprevalence rates of STDs in Uganda. It has even been postulated that the level of syphilis prevalence in a population may function as a prognostic factor for the development of HIV seroprevalence [17].

During the last decades, many efforts have been started in Uganda to fight the further spread of HIV infection, such as educational programs about viral transmission [18, 19]. In addition, a network of counseling for people living with HIV has been established [20].

As also mentioned in other studies, one limitation exists with regard to data taken from ANCS on HIV prevalence: as infertile women or women using modern contraception do not visit ANCs, these women are obviously not represented in the studies concerning HIV in pregnancy. The exclusion of this group of women may result in an underestimation of HIV prevalence in the female population because infertility is mostly caused by STDs including HIV [21–26]. Other studies have shown that fertility rates decrease with HIV infection [15]. The reduced incidence of recognized pregnancy and fertility in the earliest stages of HIV infection is consistent with a previous observation, i.e. an association between pre-existing sub-fertility and subsequent HIV infection in this cohort [27]. No data are available as to whether the women recruited for our studies had wanted to become pregnant. Many clinicians are unaware of the serostatus of the pregnant women attending an ANC without symptoms, and the extent of women's suspicions of their HIV status is unknown. Although other studies suggest that pregnancy does not involve further physiological risks of acquiring HIV-1, unprotected sex (unless in a known monogamous relationship) clearly represents a behavioral risk for HIV-1 acquisition [28].

Conclusion

In summary, the HIV prevalence rate in Uganda decreased after 1993 but remained stable at a high level between 2001 and 2007. The infection rate of syphilis, however, decreased significantly. This might be a strong predictor of decreasing prevalence rates in HIV in the coming years. There is still a lot to do regarding the prevention of HIV infection and there is a great need for health care professionals and counselors to provide services for women and men infected with HIV.

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