Prevalence and risk factors of cervicovaginal HIV shedding among HIV-1 and HIV-2 infected women in Dakar, Senegal

Karim Seck, Ngone Samb, Serge Tempesta, Claire Mulanga-Kabeya, Daniel Henzel, Papa Salif Sow, Awa Coll-Seck, Souleymane Mboup, Ibrahima N'doye, Eric Delaporte

Objectives: To assess the risk determinants and prevalence of cervicovaginal shedding of HIV-1 and HIV-2 among women in Dakar, Senegal.

Methods: We conducted a cross sectional study of 153 HIV seropositive female sex workers (FSW) and another 142 HIV seropositive women attending an infectious diseases unit, based on an interview, physical examination, and laboratory screening for major sexually transmitted infections (STI). Cervicovaginal lavage was tested for HIV-RNA by means of nested PCR. Links between cervicovaginal shedding of HIV-1 and HIV-2 and sociodemographic, clinical, and laboratory variables were identified by using odd ratios and 95% confidence intervals. Logistic regression analysis was used to identify independent links with HIV shedding.

Results: The detection rate of HIV-RNA in cervicovaginal lavage fluid was low among FSW, with no difference between HIV-1 (7/90: 8%) and HIV-2 (3/48: 6%). The rate was far higher among the other women (41%, 48/117; 33%, 7/21 for HIV-1 and HIV-2, respectively). In multivariate analysis, high plasma viral load (>40,000 copies/ml) (AOR = 2.4 (1.0–5.6) p = 0.04) and basic vaginal pH (AOR = 2.2 (1.3–3.7) p = 0.002) were independently associated with HIV-1 shedding. For HIV-2 a CD4 count < 200 cells × 10^6/l was the only factor associated with the shedding of HIV-2 (AOR = 9.0 (0.9–93)). The genital shedding rate was higher with HIV-1 than with HIV-2 (OR = 2.1 (0.9–4.8), but this difference disappeared after adjustment for the CD4+ cell count (AOR = 1.2 (0.5–2.9)).

Conclusion: Advanced disease stage and immunosuppression are the major risk determinants for shedding of both HIV-1 and HIV-2. Basic vaginal pH is also a risk determinant for HIV-1 shedding.

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Keywords: cervicovaginal shedding; HIV; Senegal

Introduction
In Sub-Saharan Africa HIV is mainly transmitted heterosexually and perinatally. Factors influencing HIV shedding in the genital tract are thus likely to be important risk determinants. It was recently shown that HIV-1 viral load correlates with the risk of sexual transmission. It is also known that factors such as advanced disease stage, CD4+ lymphocyte depletion, vitamin A deficiency, certain sexually transmitted infections (STI), and pregnancy are associated with genital shedding of HIV-1. The relative contribution of these factors could be influenced by viral, host, and/or environmental characteristics. Effective strategies to prevent both perinatal and heterosexual transmission must take into account genital shedding of HIV-1. HIV-2 appears to be less infective than HIV-1, and there have been few studies on the determinants of its genital shedding.

We conducted a cross sectional study aimed at identifying risk factors for HIV-1 and HIV-2 genital shedding among women living in Dakar, Senegal, where both viruses are prevalent.

Population and methods
STUDY POPULATION AND DATA COLLECTION
A cross sectional study was conducted in 1997 among two populations of HIV seropositive women consecutively recruited in Dakar, Senegal. The first population consisted of 153 asymptomatic female sex workers (FSW) enrolled during a routine medical visit at the Institut d’Hygiène Sociale (IHS). The second group comprised 142 women attending the infectious diseases unit of Fann hospital.

With their informed consent, the women were interviewed on their sociodemographic characteristics, sexual behaviour, obstetric history, contraceptive use, and clinical signs. A physical examination was carried out to define the clinical stage of HIV infection according to the 1993 CDC classification, and a gynaecological examination was performed. Cervicovaginal lavage fluid was collected after the sampling for STI as follows: 10 ml of phosphate buffered saline (PBS) was injected into the genital tract, and at least 5 ml of fluid was aspirated 1 minute later, then stored at −70°C until viral RNA assay.

LABORATORY PROCEDURES
Trichomonas vaginalis and Candida albicans were detected by direct microscopic examination of a wet mount and after Gram staining. The pH of the vaginal fluid was measured with commercial pH paper (pH 4.0–7.0).

Neisseria gonorrhoeae was identified by growth on modified Thayer-Martin medium. Chlamydia trachomatis antigen was detected...
with an enzyme immunoassay (EIA, Microtrack, II Syva, France). Syphilis was diagnosed by the RPR (Becton Dickinson) and TPHA tests (Fujirebio, Tokyo, Japan).

Screening for anti-HIV antibodies was performed using a rapid test (Capillus HIV-1/2, Cambridge Diagnostic, Ireland) and a line immunoassay (Innolia HIV1/2, Innogenetics, Belgium). CD4+ lymphocytes were counted by flow cytometry using a FACScan (Becton-Dickinson). The vitamin A concentration was determined by high performance liquid chromatography.

Viral load in plasma was quantified only in HIV-1 infected women, using the Amplicor HIV-1 Monitor kit, version 1.5 (Roche Diagnostic, NJ, USA).

Qualitative assay of HIV-1 and HIV-2 RNA in cervicovaginal lavage fluid was performed by nested PCR amplification of the pol region, as previously described.3

DATA ANALYSIS
Dbase 5, EPI-INFO (version 6.0, Centers for Diseases Control and Prevention, Atlanta, GA, USA), and Logistic Regression software (3.11 Ef Dallagile, Andover, USA) was used for data analysis. Proportions and rates were compared by using the χ² and Fisher’s exact test, and means were compared using Student’s t test. Variables associated with HIV cervicovaginal shedding in univariate analysis (p<0.05), and forced variables, were included in a multiple logistic regression model. Adjusted odd ratios (AORs) and 95% CIs were calculated using logistic regression parameters. The Hosmer-Lemeshow statistic was used to assess the fit of the final model.

Results
CHARACTERISTICS OF THE STUDY POPULATION
Table 1 summarises the sociodemographic data and the prevalence of major STIs in the two population groups.

All the FSW enrolled in the study were officially registered according to the Senegalese law. Their average number of clients per week was 11.0 (range 1–35). All were asymptomatic, with higher CD4+ cell counts and lower plasma viral load than the other group of women (p<0.001).

The rates of STI were low in both groups. However, 33% of the female sex workers and only 2% of the other women were seropositive for syphilis. HIV-1 infection predominated in both groups. HIV-2 infection and dual HIV-1/2 seropositivity were more frequent in the female sex workers than in the other women (p<0.001).

The prevalence of HIV RNA shedding was low among the female sex workers, with no difference between HIV-1 (7/90 (7.8%)) and HIV-2 (3/48 (6.2%)). It was far higher in the other women (41%, 48/117; 33.3%, 7/21 for HIV-1 and HIV-2, respectively); HIV-1 shedding was more frequent than HIV-2 shedding in these women (OR = 4.1 (1.6–10.9)). HIV shedding was not detected in any women with dual HIV-1/2 seropositivity (15 female sex workers, four other women). Because of the
Table 2 Univariate correlates of cervicovaginal HIV-1 and HIV-2 shedding

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV-1 shedding</th>
<th>HIV-2 shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=55)</td>
<td>Negative (n=152)</td>
</tr>
<tr>
<td>Vaginal pH (mean)</td>
<td>5.7</td>
<td>5.35</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>45/55</td>
<td>104/150</td>
</tr>
<tr>
<td>Genital ulceration</td>
<td>24/58</td>
<td>38/149</td>
</tr>
<tr>
<td>CD4 count &lt;200 × 10^6/l</td>
<td>27/45</td>
<td>35/135</td>
</tr>
<tr>
<td>Plasma viral load &gt;40 000</td>
<td>34/55</td>
<td>45/144</td>
</tr>
<tr>
<td>Serum vitamin A &lt;0.07 µmol/l</td>
<td>24/41</td>
<td>47/105</td>
</tr>
<tr>
<td>AIDS clinical stage (stage C)</td>
<td>28/53</td>
<td>17/131</td>
</tr>
</tbody>
</table>

OR = odds ratio.
CI = confidence interval.
* p test.
ND = not done.

Discussion

This study shows that the rates of cervicovaginal shedding of HIV-1 and HIV-2 among infected Senegalese women are similar after adjustment for the HIV disease stage. Few studies have addressed the risk factors for cervicovaginal HIV-2 shedding.\(^3\) We found that HIV-2 RNA was detectable by PCR in cervicovaginal lavage fluid from 14.5% of 69 HIV-2 infected women. The presence of HIV-1 and HIV-2 RNA in the genital tract was clearly associated with factors reflecting HIV disease progression such as CD4+ lymphocyte depletion; this was well known for HIV-1 but not for HIV-2. Like HIV-1, female genital HIV-2 shedding is probably associated with a high circulating viral load. Therefore, risk determinants for cervicovaginal shedding of HIV are a function of the disease progression and the viral level rather than the virus type suggesting that the low sexual transmission rate of HIV-2 reported\(^7\) could be explained by the slow disease progression, which characterised the HIV-2 infection. This is also the likely reason for the low rate of vertical transmission of HIV-2.\(^2\)

STIs are well established cofactors for sexual transmission of HIV-1. We observed a weak association between vaginal ulceration and HIV-1 shedding in the genital tract. No correlation was observed between the presence of any specific STI and cervicovaginal shedding of HIV-1 or HIV-2. This may be partly explained by the low prevalence of STIs in our population, and their treatment, as treatment of STIs reduces the prevalence of HIV-RNA detection in the female genital tract.\(^3\)

The lack of any association between cervicovaginal HIV shedding and oral contraceptive use or cervical ectopy may be due to their low prevalence in the study population, and to its small size.

Most women in our study were deficient in vitamin A, and we found no correlation with HIV shedding. The prevalence of cervicovaginal HIV-1 shedding increased with the vaginal pH (ranging from 5.0 to 5.9). Increased vaginal pH could result from bacterial vaginosis, a factor associated with susceptibility to sexual HIV transmission in women. Alternatively, traditional product for local vaginal hygiene may also increase pH.

In conclusion, our results show that advanced HIV disease stage is the main risk determinant for female genital shedding of both HIV-1 and HIV-2. HIV-1 shedding was also associated with higher vaginal pH.

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Contributors: KS was responsible for the care of the FSW, for supervising the study in Senegal, and for preparing the manuscript; NS and SM supervised laboratory testing at CHU Fann; SPS was the principal investigator in Senegal; ED was responsible for supervising the study at CHU Fann; IN was the principal investigator in Senegal; KS was responsible for the care of the FSW, for supervising the study in Senegal, and for preparing the manuscript.

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