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

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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 fluid 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 cervicovaginallavage 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 × 10⁹/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 asymtomatic 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 Dallalge, 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<0.25), 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>24/55</td>
<td>38/149</td>
</tr>
<tr>
<td>CD4 count &lt;200 × 10⁹/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.  
ND = not done.