

# Brief Original Article

# Low prevalences of HIV infection and HSV genital shedding in the general adult female population in Senegal

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#### **Abstract**

Introduction: Herpes simplex virus (HSV) is the main co-factor for heterosexual transmission of the human immunodeficiency virus (HIV) in sub-Saharan Africa, and could be involved in the dynamics of the HIV epidemic in Senegal.

Methodology: Genital shedding of HSV was evaluated in adult females who had visited the provincial healthcare centres in Diass, Louga, and Kebemer in Senegal. Study subjects were interviewed by a healthcare worker for sociodemographic characteristics and sexual behavior, and HIV serology was offered. In addition, cervical secretion lavage samples were evaluated for HSV DNA by real-time polymerase chain reaction (PCR), the melting curve analysis of which permitted distinction between HSV type 1 (HSV-1) and HSV type 2 (HSV-2).

Results: Among 302 women (mean age, 40 years) enrolled, none were infected by HIV. The mean age at first sexual intercourse was 20 years, and the mean number of sexual partners in the previous year was 1.3 (range, 1–7). Only 6 of 302 (1.9%) women had cervico-vaginal secretions positive for HSV DNA. No association between HSV DNA shedding and any sociodemographic or biological variables was found. Surprisingly, genital shedding of HSV-1 was found in two (0.7%) women, representing 33% of herpes-shedding women, and HSV-2 in four (1.5%) women.

Conclusions: Taken together, our observations indicate a low prevalence of HSV DNA genital shedding in adult Senegalese women.

**Key words:** HSV-1; HSV-2; HIV; childbearing-age women; Senegal.

J Infect Dev Ctries 2015; 9(11):1272-1276. doi:10.3855/jidc.6227

(Received 07 November 2014 – Accepted 09 July 2015)

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#### Introduction

Both biological plausibility and epidemiological evidence strongly suggest that genital herpes simplex virus (HSV) infection is a potent co-factor in the heterosexual, bidirectional transmission of human immunodeficiency virus type 1 (HIV-1) in sub-Saharan Africa [1-6]. The attributable risk for HSV type 2 (HSV-2) to HIV acquisition in sub-Saharan Africa populations ranges between 25% and 35% [7].

The current HIV epidemic in Senegal, which is basically concentrated, differs from HIV epidemics encountered in many other African countries (generalized epidemic). The prevalence of HIV-1 among female sex workers (FSW) in Dakar rapidly increased from 0.1% in 1986 to more than 10% in 1994 and 19% in 1997 [8]. By contrast, the HIV-1 seroprevalence has remained low among pregnant women with rates of 1.5% in 2004 and 0.7% in 2006

[9]. Therefore, the HIV-1 epidemic in Senegal is clearly of a concentrated type with a low and stable prevalence in the general population, but has a strong prevalence in high-risk groups. Furthermore, the HIV-2 epidemic in Senegal has remained at a low level since its beginning [9].

The low prevalence of HSV-2 in Senegal has been recently implicated as an important factor in the low rate of HIV dissemination in Senegal [10]. In a recent survey, the HIV-1 and HSV-2 seroprevalence was low, estimated at approximately 1% and 15% among pregnant women in Dakar and in Kaolack, respectively, in 2004 [10,11]. In contrast, the HSV-2 seroprevalence in men having sex with men and living in the urban areas of Senegal was much higher, reaching 23% in 2005 [12]. Furthermore, the HIV-1 and HSV-2 seroprevalence in FSW in Senegal were reported to be as high as 23% and 25%, respectively,

in 2009 [11]. These observations suggest the possibility that HSV-2 infection is a co-factor in HIV transmission in certain high-risk Senegalese populations, rendering it necessary to survey both HIV and HSV-2 epidemics from a public health perspective [10].

In many African countries where heterosexual intercourse is the major mode of HIV transmission, the risk of HIV spreading in the general population appears to be associated with the presence and amount of HIV in the genital tract, which in turn correlates with HSV DNA shedding in co-infected individuals [13-15].

The aim of the present study was to evaluate the genital shedding of HSV in the general adult female population living in Senegal.

## Methodology

Study inclusion

Adult women attending provincial healthcare centres in the cities of Diass, Louga, and Kebemer in Senegal were prospectively recruited on a volunteer basis after informed consent was obtained. The wording of this verbal consent was derived by consensus of the group of physicians and stakeholders involved in the study. None of the women studied belonged to a high-risk group for HIV infection, such as FSWs.

The three healthcare centres participate in the free medical services provided annually throughout Senegal by the Ministry of Health. Patients receive systematic medical screening, family planning, genital health evaluations including sexually transmitted infection (STI) diagnosis and care, or colposcopy for cervical cancer screening. Women enrolled in the study were interviewed by a healthcare worker for sociodemographic characteristics and sexual behavior, including age at first sexual intercourse and the number of sexual partners in the previous year. They also underwent clinical examination. Testing for HIV based on the national guidelines [11] and testing for other common etiologic agents causing STI was offered to the participants.

The study protocol, including the questionnaire and the evaluation of a French laboratory able to accurately identify HSV DNA genital shedding was reviewed and approved by the AIDS Division under the supervision of the Ministry of Health of Senegal. All individuals positive for HIV and suffering from STIs were referred to different healthcare centres throughout the country for clinical and psychosocial care, if needed.

Sample collection and processing

Blood samples were obtained by venipuncture in Vacutainer tubes (Becton Dickinson, Franklin Lakes, USA), and aliquoted plasma was kept at -80°C for HIV serology confirmation, if needed.

Cervico-vaginal secretions were collected using standardized 60-second cervico-vaginal lavage, as previously described [16]. After introduction of a speculum, the cervico-vaginal lavage was performed with 3 mL of 1 M phosphate-buffered saline, pH 7.2. All samples were processed within two hours of collection by centrifuging the fluid at 1,000 g for 10 minutes followed by separately freezing the cell-free supernatant and cell pellet at -80°C.

## Genital shedding of herpes DNA

Total DNA from cell-free and cell pellet of cervico-vaginal lavage samples was extracted and purified on a silicated column (QIAamp DNA Mini Kit, Qiagen, Courtaboeuf, France), following the manufacturer's instructions. DNase-free elution buffer (100  $\mu$ l) was added to the total extracted nucleic acids from each sample, and kept frozen at -80°C until processing. After thawing, equal volumes of extracted DNA from cell-free fluid and cell-pellet of cervico-vaginal secretions samples were mixed for further polymerase chain reaction (PCR) analysis.

HSV DNA detection and quantification was performed by standardized real-time PCR, as described previously [17]. The real-time PCR for HSV used the HSVpolF and HSVpolR primer set, allowing amplification of a 140 bp product [18]. A pair of fluorescently labeled probes, HSV-2 FLU and HSV-2 LCR, were used for real-time quantification [17]. The number of β-globin copies in each specimen was also quantified by the Light Cycler Control Kit DNA (Roche Molecular Systems, Inc., Branchburg, USA) [17]. The normalized value of the HSV DNA load the corresponding to ratio ([HSV copies number/albumin copies number] x 2 x 10<sup>6</sup>) was expressed as the number of HSV DNA copies per 10<sup>6</sup> cells. The threshold of detection of real-time PCR for HSV DNA is 10 copies/10<sup>6</sup> cells (data not shown); thus, the lower limit of detection of HSV DNA is 100 copies/10<sup>6</sup> cells in cervico-vaginal lavage sample, taking into account the 1:10 dilution introduced by the lavage procedure [16].

At the end of the cycles obtained by real-time PCR assay, a melting curve was generated to discern HSV-1 from HSV-2, which have different melting temperatures, as previously described [17,19,20].

#### Statistical analysis

Data were entered into Epi-info version 6.0. Frequency of distributions, percentages, mean, and median were used to describe variables. Odds ratios (with 95% confidence intervals [CIs]) were used to measure the magnitude of the associations between socio-demographic (age groups and sexual behaviours) and biological (serostatus for HIV; HSV-1/HSV-2 DNA genital shedding) variables. A p value < 0.05 was considered significant.

#### Results

#### Study population

A total of 302 adult women were enrolled in the study in 2011. The mean age was 40 years (range, 18–75). The majority were married (90%), mainly with a monogamous matrimonial status (58%); a minority of cases were polygamous (42%). The education level was primary (59%) or secondary (38%), and few women had achieved higher education (3%). The mean age at first sexual intercourse was 20 years (range, 10–35). The mean number of sexual partners in the previous year was 1.3 (range, 1–7). The seroprevalence of HIV was 0% in this series.

# Genital shedding of herpes DNA

All study cervico-vaginal wash samples were positive for  $\beta$ -globin detection by real-time PCR, confirming both the correct quality of extracted DNA and the sensitivity to detect and quantify genital HSV DNA.

HSV DNA was detected in only 6 of 302 (1.9%) of participants. The mean genital herpes load in the 6 women shedding HSV DNA was 7.2 10<sup>5</sup> copies/10<sup>6</sup> cells (range, 1.4 10<sup>2</sup>–4.2 10<sup>6</sup>). Demographic and biological characteristics of study women by genital HSV shedding groups are depicted in Table 1. No association between HSV DNA shedding and any sociodemographic or biological variable was found.

The mean age of study women shedding HSV DNA was 28 years (range, 18–43). All were married (100%), and most were monogamous (4/6, 67%), but two (33%) were polygamous. Education level was low in three (50%), mid-level in two (33%), and advanced in only one (17%) subject. The mean age at first sexual intercourse was 20 years (range, 14–25). All women had had only one sexual partner in the previous year. All women shedding HSV DNA were seronegative for HIV.

**Table 1.** Genital herpes shedding in 302 adult Senegalese women attending healthcare centers in Senegal by their mean sociodemographic characteristics and their HIV serostatus.

	Negative genital HSV shedding <sup>£</sup> $(n = 296)$	Positive genital HSV shedding $(n = 6^{\mu})$	Odds ratio*	p value <sup>\$</sup>
Age (mean [range], years)	41 (15–75)	28 (18–43)		
< 40 (n, %)	137 (46%)	5 (83%)	5.8 (0.6–132.8)	0.07
≥ 40 (n, %)	159 (54%)	1 (17%)	0.2 (0.0-1.5)	0.07
Matrimonial status (n, %)				
Unmarried	31 (10%)	0 (0%)	0.0 (0.0-8.3)	0.4
Married (monogamous)	141 (48%)	4 (67%)	2.2 (0.4–18.0)	0.3
Married (polygamous)	125 (42%)	2 (33%)	2.2 (0.1–4.4)	0.7
Education level* (n, %)				
High	8 (3%)	0 (0%)	0.0 (0.0-41.0)	0.7
Middle	111 (37%)	3 (50%)	1.7 (0.3–10.7)	0.5
Low	178 (60%)	3 (50%)	2.1 (0.2-52.8)	0.5
Age at first sexual intercourse (mean [range], years)	20 (10–35)	20 (14–25)		
10–17 (n, %)	92 (31%)	2 (33%)	1.1 (0.1–7.2)	0.9
18–35 (n, %)	204 (69%)	4 (67%)	0.9(0.1-7.2)	0.9
Number of sexual partners in the previous year (n, %)				
0-1	208 (69%)	6 (100%)	NA	0.2
$\geq 2$	66 (22%)	0 (0%)	0.0(0.0-3.1)	0.2
HIV serostatus (n, %)	. ,		. ,	
Negative	296 (100%)	6 (100%)	NA	NA

<sup>&</sup>lt;sup>£</sup> Genital HSV shedding was assessed by in-house real-time PCR in the acellular part of cervico-vaginal washing samples, as described [20]; the threshold of detection of real-time PCR for HSV DNA is 10 copies/10<sup>6</sup> cells, and the lower limit of detection of HSV DNA is 10.0 log10/10<sup>6</sup> cells in cervico-vaginal lavage sample, taken into account the 1:10° dilution of genital secretions provided by the washing procedure [19]; <sup>μ</sup> Among the 6 women shedding HSV, 4 (67%) were shedding HSV-2 and 2 (33%) HSV-1, according to the analysis of the melting curve obtained at the end of the cycles by real-time PCR assay; \* 95% confidence interval in brackets; <sup>§</sup> Statistical analysis used the software was Epi Info version 6.0; NA: not applicable

Among the six women shedding HSV, four (67%) had HSV-2, and two (33%) had HSV-1. The two women shedding HSV-1 DNA were 33 and 21 years of age, respectively; both were married in polygamous marriages and had a secondary or higher education level.

#### **Discussion**

In this study, Senegalese women in the general population had very low rates of HIV and HSV infection. Among women not belonging to a high-risk group for HIV infection, we found the seroprevalence of HIV to be zero, and less than 2% were shedding HSV DNA in genital secretions. Surprisingly, among those shedding HSV, one-third were positive for HSV-1, while two-thirds were positive for HSV-2. Women with cervico-vaginal secretions positive for HSV DNA shedding did not demonstrate any significant sociodemographic or biological trends compared to women who had negative secretions. Although the results have to be interpreted cautiously because of limited data and possible selection bias, some relevant points merit discussion.

The present observations confirm and extend our previous serosurveys from the general population of adult Senegalese women living in Dakar and Koalack, who had low rates of seropositivity for HSV-2 infection [10,11], comparable with those usually reported in Western countries [21]. The low prevalence of HSV-2 in adult Senegalese women contrasts with the higher HSV-2 seroprevalence reported in the general populations living in neighbouring countries [22-24], as well as in central or southern Africa [25-29]. The low rate (1.3%) of women shedding HSV-2 DNA in our study population clearly reflects the low HSV-2 seroprevalence of this population, since only a minority of HSV-2-infected individuals usually shed herpes DNA in their genital secretions [30]. Furthermore, no association was found in our study population between HSV-2 DNA shedding and HIV infection. These low rates of HSV DNA genital shedding provide biological plausibility and rationale suggesting that HSV-2 infection does not represent a significant co-factor for HIV transmission in the general adult Senegalese population, in contradiction to the close relationship between HSV-2 and HIV-1 infections in many sub-Saharan countries where HIV epidemic is generalized [25,31]. Taken together, our observations confirm that the HIV as well as HSV-2 epidemics have not yet spread through the general adult Senegalese population.

Surprisingly, genital shedding of HSV-1 was found in only two (0.7%) women or one-third of HSV DNA shedding women. This finding suggests that orogenital transmission of HSV-1 does occur in Senegal and the pattern of herpes transmission may be changing in the Senegalese population. Indeed, the classical pattern of HSV-1 and HSV-2 infections associated with oral or genital diseases, respectively, remains the rule in certain regions of the world such as sub-Saharan Africa, where HSV-1 infection remains a nearly ubiquitous infection of childhood, and HSV-2 infection is an STI among adults [24,32]. In contrast, the differentiation of HSV-1 from HSV-2 based on anatomical site of infection is far from absolute in developed countries. Genital herpes is frequently caused by HSV-1 due to the delay in the acquisition of oral HSV-1 infection among children developed countries, rendering a significant proportion of young adults susceptible to genital HSV-1 infection at initiation of sexual activity [33,34]. Furthermore, it is well recognized that a clear HSV type-related tropism might be limited to the permissiveness of the orofacial region for HSV-1, and that both HSV-1 and HSV-2 serotypes may readily establish infections below the neck [35].

#### **Conclusions**

Our present observations in adult Senegalese women suggest that the classical pattern of HSV-1 and HSV-2 infections associated, respectively, with oral or genital diseases may be changing in Senegal, as previously observed in several Western countries [33,36] and more recently in Thailand [24]. If this hypothesis is correct, the seroprevalence of HSV-1 may decrease in young adults living in Senegal, whereas the rate of primary genital herpes caused by HSV-1 could increase. Such a change would be consistent with observations from Guinea-Bissau, a neighbouring country of Senegal, in which the shedding of genital herpes was 1.4% for HSV-1 and 7.7% for HSV-2 among women attending sexual health clinics in Bissau [37]. Further study in Senegal and Western Africa is needed to confirm this latter hypothesis.

# **Acknowledgements**

We express our gratitude to the Senegalese Ministry of Health and the National AIDS and STIs Division. We thank Dr Thomas V Brogan, Seattle Children's Hospital, University of Washington, Seattle, WA, USA, for reviewing the English of the manuscript. This work was supported by the Senegal's National AIDS Program in the National AIDS and STIs Division.

#### **Authors' contributions**

CTK and LB conceived and designed the research. SD carried out molecular analyses with the contribution of MM and SBG. HDN and AD initiated, supervised, and coordinated the study in Senegal. LB and MM contributed materials and quality control for analysis in Paris. SD, LB, SM, HDN, and CTK drafted the manuscript. All authors read and approved the final manuscript.

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Conflict of interests: No conflict of interests is declared.