

HSV-2 Seroprevalence and Seroincidence in an Ethnically Diverse Cohort of HIV-1 Infected Persons



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Abstract

Objectives: To determine HSV-2 seroprevalence at HIV-1 diagnosis and seroconversion at HIV-1 diagnosis, and associated risk factors, in an ethnically diverse cohort.

Methods: HSV type-specific antibodies were detected by enzyme immunoassay and immunoblot.

Results: The cohort comprised 850 adults diagnosed HIV-1 positive in 1986-2001, including 534 (63%) males, 467 (55%) heterosexuals, 338 (40%) homosexuals, and 385 (45%) black-African, 371 (43%) white, and 83 (10%) black-Caribbean patients. HSV-2 seroprevalence at HIV-1 diagnosis was 537/850 (63%). Seroconversion was associated with female gender, heterosexual risk group, black ethnicity and older age. Patients remained in follow-up for median 3 years (range 1-16 years). Among 123 persons randomly selected among those who were HSV-2 seronegative at HIV-1 diagnosis, seroconversion occurred in 12/123 (10%), at median 4 years after HIV-1 diagnosis. HSV-2 seroconversion was associated with a diagnosis of other sexually transmitted diseases (STDs) including human papilloma virus infection (p=0.005) and gonorrhoea (p=0.05). Overall, 116/549 (21%) HSV-2 seropositive patients received a clinical diagnosis of genital herpes; 46/116 (40%) had a virological diagnosis by virus culture. A diagnosis of genital herpes was at least four times more likely in HSV-2 seropositive patients who tested HIV-1 positive before 1997 (p=0.0001).

Conclusions: HSV-2 seroprevalence was high at the time of HIV-1 diagnosis, confirming the epidemiological association between the two infections. HSV-2 seroconversion was strongly associated with other STDs and therefore a marker of high-risk sexual behaviour. Although most HSV-2 infections remained undiagnosed by routine clinical care, the likelihood of developing genital HSV-2 disease declined significantly after 1996.

Background

- HSV-2 seropositivity is a marker of genital HSV infection and is associated with both symptomatic and asymptomatic shedding of HSV-2 in the genital tract¹.
- HSV-2 infection is highly prevalent worldwide^{2,3}, although most infected persons are either asymptomatic or, more commonly, have genital symptoms that remain unrecognised⁴. Despite the absence of recognised disease, most HSV-2 seropositive persons shed virus as some time and are potentially infectious⁵.
- HSV-2 seroprevalence varies between regions and populations and the risk factors for infection are numerous and diverse⁶.
 - **General population:**
 - * US (12 years) 22%⁶
 - * Europe (>12 years): 4%-24%⁶
 - * Sub-Saharan Africa (adults): 20%-80%⁶
 - **HIV-1 positive cohort:**
 - * Europe: men 38-54%, women 17-66%
 - * US: heterosexual men 63%, homosexual men 66%, heterosexual women 75-78%
 - * Barbados: 77%
 - * Sub-Saharan Africa: >80%
- There is a strong epidemiological association between HSV-2 and HIV-1 infection⁷
 - Common risk factors for infection
 - Bi-directional biological interaction between the two viruses
- In prospective studies, HSV-2 seropositivity increased the risk of HIV-1 acquisition and transmission by approximately two-fold⁸. The relationship appears to be independent of a clinical diagnosis of genital herpes.
- HSV-2 infection may promote HIV-1 shedding in the genital tract and increase HIV-1 RNA levels in blood^{9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100}.

Objective

The aim of this study was to determine the rates of HSV-2 seroprevalence and seroconversion and the natural history of HSV-2 infection in an ethnically diverse cohort of adults who were diagnosed HIV-1 positive between 1986 and 2001.

Materials and Methods

Study population

- 850 persons diagnosed HIV-1 antibody positive in 1986-2001 under went retrospective testing for HSV-2 antibodies using stored sera collected at the time of HIV-1 diagnosis.
- A subset of 123 patients chosen randomly among those who tested HSV-2 seronegative underwent further serological testing at ≥ 1 year after HIV-1 diagnosis.
- Clinical and laboratory records were reviewed retrospectively to determine the occurrence of a clinical and virological diagnosis of genital herpes, and the diagnosis of other STDs.

HSV type-specific serology

HerpeSelect IgG EIA (Focus Technologies, Cypress, California, US). Equivocal (0.9-3.0) and positive (>3.0) EIA results retested by HerpeSelect Immunoblot IgG (Focus Technologies), and with sera of persons from Uganda or Kenya by inhibition-EIA, preincubating the sera with HSV-1 and HSV-2 virus lysates²¹.

Statistical Analysis

Univariable analysis for χ^2 or Fisher's exact tests for qualitative variables and Mann-Whitney U tests for quantitative variables. Multivariable logistic regression analysis. (SAS v8).

Results

Study population

Between Jan 1986 and Dec 2001 (median 1999), 850 adults with a median age of 33 years (range 17-71 years) were diagnosed with HIV-1 infection. Their demographic characteristics are summarised in **Table 1**.

- Black African patients: 351/385 (91%) born in sub-Saharan Africa (predominantly Uganda, Zimbabwe, Zambia, Nigeria, Ivory Coast, Ghana, Kenya, Ethiopia and Sierra Leone), 24/385 (6%) born in the UK and 10/385 (3%) born in other European countries.
- White patients: 263/371 (71%) born in the UK, 82/371 (22%) born in other European countries, and 26/371 (7%) born in the US or Australia.
- Black-Caribbean patients: 44/83 (53%) born in the UK and 39/83 (47%) born in the Caribbean (predominantly Jamaica).
- Remaining 11 patients: Indian sub-continent (8), Middle East (2), North Africa (1).
- Patients remained in follow-up for median 3 years (range 1-16 years).

HSV-2 seroprevalence at the time of HIV-1 diagnosis

- HSV-1 seroprevalence: 752/850 (88%); 95% CI: 86%-91%
- HSV-2 seroprevalence: 537/850 (63%); 95% CI: 60%-66%.
- HSV-2 seroprevalence increased with age (**Figure 1**). Overall, HSV-2 seropositive persons were slightly older (median age 34 years, range 17-71 years) than those with a negative HSV-2 antibody result (median age 32 years, range 17-70) (p=0.01).
- The median year of HIV-1 diagnosis was 1999 in both HSV-2 seropositive persons (range 1986-2001) and HSV-2 seronegative persons (range 1990-2001) (p=0.06).
- HSV-2 seroprevalence varied significantly according to gender, risk group, and ethnicity. (**Table 2**). Due to the fact that most heterosexual persons were women, a possible confounding association between gender and risk group could not be excluded. Separate multivariable models were applied to the two variables (**Table 3**). In both models, a positive HSV-2 antibody result remained strongly associated with black ethnicity and older age at the time of HIV-1 diagnosis. After controlling for these variables, females (model 1) and heterosexuals (model 2) were both more likely to have a positive HSV-2 test result than males or homosexuals.

- The overall prevalence of HSV-2 antibody was significantly higher among persons born outside of the UK (372/519, 72%) than those born in the UK (165/331, 50%) (p<0.0001).
- There was no association between HSV-2 seropositivity and HSV-1 serostatus (**Table 2**).

HSV-2 seroconversion after HIV-1 diagnosis

- 123 HSV-2 seronegative patients (randomly selected)
 - 98/123 (80%) males, 80/123 (65%) heterosexuals, 43/123 (35%) heterosexuals, 87/123 (71%) whites, 25/123 (20%) black-Africans, and 11/123 (9%) black-Caribbeans.
 - HIV-1 diagnosis: 1990-2000 (median 1997).
 - Median age: 32 years (range 17-70).
 - Median follow-up: 5 years (range 2-12 years).
- HSV-2 seroconversion occurred in 12/123 (10%) at median 4 years (range 2-11 years) after HIV-1 diagnosis.
- HSV-2 incidence rate: 1.8 cases/100 person-years (95% CI: 0.8-2.8).
 - Those who seroconverted included 11/98 (11%) males and 1/25 (4%) females, 7/80 (9%) heterosexuals and 5/43 (12%) homosexuals, and 9/87 (10%) white and 3/25 (12%) black-African patients.
 - Among the same 123 patients, 101 (82%) were tested for other STDs at median 2 time-points (range 1-12), following standard clinical protocols.
 - 54/123 (44%) received a diagnosis of one (n=38), two (n=15) or three (n=1) STDs, including HPV (45/123, 37%), gonorrhoea (13/123, 11%), chlamydia (6/123, 5%), acute syphilis (3/123, 2%), and other infections (acute hepatitis B, n=2; acute hepatitis A, n=1; scabies, n=1) (**Table 4**). The diagnosis of HPV infection was based on the detection of genital warts in 39/45 (87%) patients and the detection of cytological abnormalities of cervical or anal smears in the absence of visible genital warts in 6/45 (13%) patients.
 - A significant association was found between HSV-2 seroconversion after HIV-1 diagnosis and a diagnosis of HPV infection (p=0.005), gonorrhoea (p=0.05) or other infections (acute hepatitis A, acute hepatitis B or scabies, p=0.0001). Other predictors of HSV-2 seroconversion could not be identified due to small numbers.

Clinical and virological diagnosis of genital herpes

- A clinical diagnosis of genital herpes was made in 116/549 (21%) HSV-2 seropositive patients. None of the 12 HSV-2 seroconverters received a diagnosis of genital herpes.
- Predictive factors for a clinical diagnosis of genital herpes in univariable analyses were male gender, non heterosexual risk group and white ethnicity (**Table 5**).
- The year of HIV-1 diagnosis was significantly different in HSV-2 seropositive patients who received a clinical diagnosis of genital herpes (median 1997, range 1990-2001) compared with HSV-2 seropositive patients who remained undiagnosed clinically (median 2000, range 1986-2001) (p=0.0001). Overall, 63/140 (45%) persons diagnosed HIV-1 positive before 1997 had a clinical diagnosis of genital herpes compared with 42/397 (11%) patients diagnosed from 1997 onwards (p=0.0001). Multivariable logistic regression analysis confirmed the strong correlation between pre-1997 HIV-1 diagnosis and a clinical diagnosis of genital herpes: adjusted odds ratio (OR) 5.11; 95% CI 3.28-7.98; P=0.0001. In addition, in multivariable analysis there remained a significant association between heterosexual risk group and lack of a clinical diagnosis of genital herpes: OR 0.55; 95% CI 0.35-0.86; P=0.0001.
- No association was found between the clinical diagnosis of genital herpes and HSV-1 serostatus (**Table 5**).
- The median age at HIV-1 diagnosis was 33 years (range 18-70) in HSV-2 seropositive patients with a clinical diagnosis of genital herpes and 34 years (range 17-71) in those without a clinical diagnosis (p=0.79).
- Of those with a clinical diagnosis of genital herpes, 46/116 (40%) yielded an HSV-2 positive genital swab by virus culture (**Table 5**). In univariable analyses, the year of HIV-1 diagnosis was the only significant predictor of a positive swab. The median year of HIV-1 diagnosis was 1996 (range 1990-2001) in patients with a positive swab and 1999 (range 1991-2001) in patients without a virological diagnosis (p=0.0006). Those diagnosed prior to 1997 were four times more likely to have a positive HSV-2 swab than those diagnosed subsequently: OR 4.01; 95% CI 1.78-9.02; p=0.0008.
- Median age at HIV-1 diagnosis was 31.5 years (range 18-70) in patients with a positive swab and 34 years (range 20-53) in those without a virological diagnosis (p=0.10).

Table 1. Demographic characteristics of 850 persons diagnosed HIV-1 antibody positive between 1986 and 2001.

Characteristics	Number (%)	
Gender	Male	534 (63)
	Female	316 (37)
Risk group	Heterosexual	467 (55)
	Homosexual	338 (40)
	IVDU*	39 (4)
	Other†	6 (1)
	Black-African	385 (45)
Ethnicity	White	371 (44)
	Black-Caribbean	83 (10)
	Other	11 (1)

*IVDU: intravenous drug use; †Other risk group: exposure to contaminated blood or blood products.

Figure 1. HSV-2 seroprevalence at HIV-1 diagnosis, stratified by age

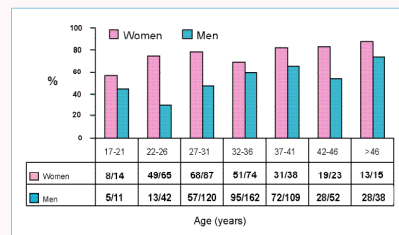


Table 2. Predictive factors for HSV-2 seropositivity at the time of HIV-1 diagnosis, identified by univariable analyses.

Factors	Number (%)	HSV-2 seropositive	P-value
Gender	Male	298 (56)	0.0001
	Female	239 (76)	
Risk group	Homosexual	167 (49)	0.0001
	Heterosexual	351 (75)	
	IVDU*	17 (44)	
	Other†	2 (33)	
	Black African	300 (78)	0.0001
Ethnicity	White	181 (49)	
	Black Caribbean	53 (64)	
	Other	3 (27)	
HSV-1 serostatus	Positive	479 (64)	0.45
	Negative	58 (59)	

*IVDU: intravenous drug use; †Other risk group: exposure to contaminated blood or blood products.

Table 3. Predictive factors for HSV-2 seropositivity at the time of HIV-1 diagnosis, identified by multivariable logistic regression analyses.

	Odds ratio	95% CIa	P-value
Model 1: Including Gender			
Female	1.51	1.03-2.20	0.03
White/other	1	NA*	NA*
Black-African	3.25	2.25-4.71	0.0001
Black-Caribbean	1.84	1.12-3.04	0.02
Age (per 5 years older)	1.18	1.08-1.30	0.0004
Model 2: Including Risk group			
Heterosexual	1.62	1.02-2.57	0.04
White/other	1	NA*	NA*
Black-African	2.70	1.64-4.43	0.0001
Black-Caribbean	1.66	0.98-2.81	0.06
Age (per 5 years older)	1.16	1.06-1.27	0.001

*CI: Confidence Interval; *NA not applicable

Table 4. Occurrence of sexually transmitted infections and HSV-2 seroconversion during median 5 years of clinical follow-up among 123 HIV-1 positive persons, according to gender and sexual orientation.

	HPV* (n=45)	Gonorrhoea (n=13)	Chlamydia (n=6)	Acute syphilis (n=3)	Other† (n=4)
Males	37	13	2	2	4
	8	0	4	1	0
Females	8	0	4	1	0
	31	13	1	1	3
Heterosexuals	14	0	5	2	1
	10	4	0	0	4

*HPV: Human papilloma virus infection, including genital warts and HPV-related cytological abnormalities in cervical or anal smears; †Hepatitis B (n=2), Hepatitis A (n=1), Scabies (n=1); significantly associated with HSV-2 seroconversion (p<0.05)

Table 5. Predictive factors for a clinical and virological diagnosis of genital herpes among HSV-2 seropositive individuals, identified by univariable analyses.

Factors	No. (%) with clinical diagnosis of genital herpes	P-value	No. (%) with HSV-2 positive swab†	P-value	
Gender	Male	76 (25)	0.02	26 (34)	0.15
	Female	40 (17)		20 (50)	
	Homosexual	50 (30)	0.005	16 (32)	0.33
Risk group	Heterosexual	61 (17)	0.28	28 (46)	
	Other	5 (26)		2 (50)	
	White	54 (30)	0.003	18 (33)	0.43
Ethnicity	Black-African	55 (18)		25 (45)	
	Other	7 (12)		3 (43)	
	Positive	101 (21)	0.51	41 (41)	0.80
HSV-1 serostatus	Negative	15 (26)		5 (33)	

*Among those with a clinical diagnosis

Conclusions

- This study was the largest to date to determine the rates of HSV-2 seropositivity among newly diagnosed HIV-1 positive patients in a European centre. The systematic approach to testing avoided selection bias.
- The overall prevalence of HSV-2 antibodies was 63% in patients who received a diagnosis of HIV-1 infection between 1986 and 2001. The prevalence was significantly higher than that observed in the general population in England and Wales (>12 years: 4%; >16 years: 10%⁶) and in STD clinic attendees in London (23-26%)^{4,30}, confirming the strong epidemiological association between HSV-2 and HIV-1.
- Consistent with previous findings³¹, HSV-2 seropositivity increased with increasing age and was significantly associated with female gender.
- A positive HSV-2 antibody result was strongly associated with black ethnicity. This finding is in line with previous studies in HIV-negative cohorts^{13,31,31}. A large proportion of persons of black ethnicity originated from sub-Saharan Africa or Jamaica and migrated to the UK in adult life. Their high HSV-2 seropositivity rates were in agreement with the high HSV-2 seroprevalence found in the countries of origin.
- Heterosexual risk group was a strong predictor of HSV-2 seropositivity, although due to the relative small number of heterosexual males a possible confounding association with gender could not be excluded.
- In agreement with other studies⁴, there was no evidence for an association between HSV-1 serostatus and either HSV-2 seroprevalence, or the clinical diagnosis of genital herpes in HSV-2 seropositive persons.
- Most HSV-2 infections remained clinically unrecognised, especially in heterosexuals.
- HSV-2 seropositive patients diagnosed HIV-1 positive from 1997 onwards were at least four times less likely to receive a clinical and virological diagnosis of genital herpes. As CD4 counts influence the clinical expression of genital herpes³², this is likely to reflect the beneficial effects of HAART.
- Persons diagnosed with HIV-1 continued to be at risk of acquiring HSV-2 despite regular counselling on risk-reduction.
- HSV-2 seroconversion was strongly associated with a diagnosis of other STDs.
 - There was a high incidence of genital warts and HPV-associated cytology abnormalities in cervical and anal smears among those who seroconverted for HSV-2 antibodies.
 - HSV-2 seroconversion was significantly associated with cases of acute gonorrhoea, acute hepatitis A or hepatitis B, and scabies, all but one occurring among homosexual males.
 - HSV-2 seroconversion after HIV-1 diagnosis may be used as a surrogate marker for high risk sexual behaviour in HIV-1 infected individuals.
 - With evidence for a bi-directional interaction between HIV-1 and other STDs, there remains an urgent need for HIV-1 prevention strategies, education and counselling.

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