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Prevalence and association between herpes simplex virus types 1 and 2-specific antibodies in attendees at a sexually transmitted disease clinic

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Background Seroprevalence of herpes simplex virus type 1 (HSV-1) and HSV-2 was determined in 1993 and 1998 in a randomly selected study group of 1024 and 654 attendees, respectively, at the sexually transmitted disease (STD) clinic of the University Hospital Rotterdam-Dijkzigt, The Netherlands. Correlations of HSV-1 and HSV-2 seropositivity were investigated. The relationship between HSV-1 and HSV-2 antibodies was also studied.

Methods Data were collected in a cross-sectional study from February 1993 until February 1994 and from January 1998 until December 1998. Glycoprotein G (gG) HSV type specific serum IgG was determined.

Results Seroprevalence of HSV-1 was 68% versus 59% (1993 versus 1998, χ^2-test $P < 0.001$), of HSV-2 it was 30% versus 22% (1993 versus 1998, χ^2-test $P < 0.001$). Using logistic regression analyses, HSV-1 and HSV-2 seropositivity were significantly associated with age and ethnicity in both groups. In 1993, HSV-1 seropositivity also correlated with lower level of education and female gender, whereas in 1998 it correlated with 'number of sexual partners in the past 6 months' and 'present diagnosis of STD'. In both groups, HSV-2 seropositivity was also more prevalent in females and related to sexual lifestyle variables. In an exposure-disease model, HSV-1 seropositivity was not correlated with HSV-2 seropositivity (odds ratio 1993 = 1.1, 95% CI : 0.8–1.7; odds ratio in 1998 = 1.0, 95% CI : 0.5–1.8).

Conclusions Seroprevalence of HSV-1 and HSV-2 is falling among STD clinic attendees in Rotterdam. A changing pattern of risk factors for HSV-1 seropositivity indicates increasing sexual transmission of HSV-1. Seropositivity for HSV-2 correlated with known risk factors. A previous HSV-1 infection does not reduce susceptibility to subsequent genital HSV-2 infections.

Keywords Herpes simplex virus type 1, herpes simplex virus type 2, genital herpes, epidemiology, seroprevalence, risk factors, transmission

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Infection with herpes simplex virus (HSV) type 1 or 2, is the most common cause of genital ulceration in the developed world.[1] Although the majority of cases are caused by HSV-2 infection, HSV-1 has been reported in an increasing number of genital herpes cases in the UK.[2] Accumulating reports have provided evidence that HSV-2 seroprevalence is on the rise, particularly in the US.[3] It was suggested that this increase was related to a lower rate of HSV-1 infection in adolescents.[4] Considering that HSV-induced genital ulcerative disease may facilitate both transmission and acquisition of human immunodeficiency virus (HIV),[5–7] it is not surprising that genital herpes has been labelled as 'a persistent health care problem, which calls for continuing public awareness'.[8] However, reliable data on HSV seroprevalence remain sketchy.

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In the Netherlands, data on HSV-2 seroprevalence are only available for sexually transmitted disease (STD) clinic attendees in Amsterdam (1986–1988) and Groningen (1998),[9,10] with a seroprevalence of 32% and 22%, respectively. It is difficult to interpret these HSV-2 seroprevalences because populations from different geographical areas were studied. Moreover, HSV-1 seroprevalence was not mentioned, despite the fact that sexual transmission of HSV-1 was suggested as increasing in recent years.[11]

In the present study, HSV-1 and HSV-2 seroprevalence was determined using HSV type-specific commercial tests[12] in randomly selected groups of patients attending our STD clinic in 1993 and 1998. Herpes simplex virus type-specific tests (a rapid immunoblot assay [RIBA] in 1993 and an enzyme linked immunosorbent assay [ELISA] in 1998) were used and correlations between demographic characteristics and sexual behaviour studied. The relationship between HSV-1 and HSV-2 seropositivity is also described.

Methods

Study population

A representative random sample was taken retrospectively from attendees visiting the STD clinic from February 1993 until February 1994 (study group 1993) and from January 1998 until December 1998 (study group 1998). Stored serum samples were selected randomly from attendees who participated in HIV serosurveillance studies at the STD clinic. The result of the HIV test was conveyed to the patient upon request (in 1998 only). All attendees were asked to answer questions covering demographic characteristics (age, ethnic group, level of education and residence) and sexual behaviour (sexual preference, having had passive anal intercourse during the past 6 months, worked as or visits to a commercial sex worker during the past 6 months, number of partners during the past 6 months and having a history of STD). The medical history was taken by a physician after which all patients underwent a routine STD check-up with examination for gonorrhoea, *Chlamydia trachomatis* infection, non-specific urethritis, vaginal trichomoniasis, condylomata acuminata, pubic lice and genital scabies, syphilis and hepatitis B. In the case of genital ulcers or erosions additional testing for herpes genitalis, syphilis and chanchroid was carried out.

The local Medical Ethics Committee of the University Hospital Rotterdam-Dijkzigt approved the study protocol and informed consent was obtained from all patients.

Laboratory methods

Sera collected from February 1993 to February 1994 were examined for HSV type-specific antibodies using a rapid immunoblot assay (RIBA) (Chiron Corporation, Emeryville, CA, USA) according to the manufacturer's guidelines. Briefly, sera were incubated on strips coated with HSV type-specific glycoprotein G (gG1 and gG2)—including a positive and negative IgG control—for 4 hours at room temperature, diluted and washed three times. Bound antibodies were detected using an anti-human specific IgG conjugate. Results were scored for HSV-1 and HSV-2 according to the manufacturer's criteria.

Sera collected from January 1998 to December 1998 were examined for HSV type-specific antibodies using a commercial ELISA (Gull Inc., Murray, UT, USA), according to the manufacturer's guidelines. Briefly, diluted patients' sera were incubated with affinity purified gG1 or gG2 antigen bound to ELISA plate wells. Plates were washed and incubated with enzyme labelled anti-human IgG to detect bound antibodies. After washing and adding a chromogenic substrate (sodium azide), and stopping reagent (sodium hydroxide), specimens containing either HSV-1 or HSV-2 antibodies produced a colour endpoint reaction which was detected with a standard ELISA plate reader. Results were scored for HSV-1 and HSV-2 antibodies according to the manufacturer's criteria. The sensitivity and specificity of the RIBA versus the Gull ELISA was 99.2% versus 99.7% and 97.1% versus 96.7%, respectively.[13,14]

Statistical analyses

To examine the relationship between demographic and sexual behaviour variables and seropositivity for HSV-1 and HSV-2, univariate analyses were performed by calculating odds ratios (OR) and 95% CI.[15]

Multivariate models were constructed with 'year of obtaining a blood sample' (1993 versus 1998) as dependent variable. Either HSV-1 or HSV-2 serostatus and all variables from univariate analyses were included in the latter model as potential confounding factors.

To determine risk factors independently correlated with HSV-1 or HSV-2 seropositivity, data were analysed in multivariate models with HSV-1 or HSV-2 serostatus as dependent variable. All variables from univariate analyses were included in these models, except HIV serostatus because there were too few seropositive samples. The variables 'sexual preference by gender' and 'having had passive anal intercourse during the past 6 months' were combined in the analyses to estimate a possible increased risk for HSV infection because of receptive anal intercourse, stratified by gender, as has been described.[9] By using stepwise backward elimination based on the likelihood-ratio test, initial predictive models were constructed with only those variables which, adjusted for each other, were significantly associated with HSV-1 or HSV-2 seropositivity. The likelihood-ratio test ($P < 0.05$) was used to eliminate variables from the initial models and the score statistic ($P < 0.1$) was used to determine whether eliminated variables could be re-entered in the reduced models. Variables significantly correlated with the dependent variable in only one year were re-introduced in the model of the other year. The remaining eliminated variables were tested either separately or all together to determine whether they could be re-entered in the reduced models.

The independent relationship between HSV-1 and HSV-2 seropositivity was studied in an exposure-disease model which was constructed following a modelling strategy described by Kleinbaum.[16] The exposure variable in this model was the test result of HSV gG1, based on the assumption that HSV gG1 seropositivity reflects prior infection with HSV-1. Likewise, the disease variable was the test result for HSV gG2. To estimate the effect of HSV-1 seropositivity on HSV-2 seropositivity as accurately as possible all variables from univariate analyses were included in this model in order to adjust for possible confounding factors. Interaction terms between the exposure variable and the main effect variables from univariate analyses were included. A more parsimonious model was constructed using the stepwise backward elimination procedure as described

previously. Separate models were constructed for both study groups. The goal of constructing these models was to calculate an overall estimate for the effect of HSV-1 seropositivity on the likelihood of HSV-2 seropositivity, for each year separately.

Results were analysed using SPSS 8.0 (SPSS Inc., Chicago, IL, USA). Other statistical tests were carried out as described in the text.

Results

From February 1993 to February 1994, 2701 (87%) patients at the STD clinic eligible to participate in the HIV serosurveillance study agreed to do so. From January 1998 to December 1998 the response rate was 78% (2904/3705). Details of non-participants in 1993 were reported elsewhere.[17] Briefly, in multivariate analyses, male and female non-participants were more likely to be foreigners. Male non-participants were more likely to have more than one partner during the past 6 months and one or more STD.

We tested a random sample of 1024 sera (403 women, 538 heterosexual men, and 83 homo/bisexual men) collected from February 1993 to February 1994. The median age (range) of women was 27 (15–63) years, of heterosexual men 33 (17–69) years and of homosexual men 34 (17–68) years. A random sample of 654 sera (292 women, 307 heterosexual men, and 55 homo/bisexual men) collected from January 1998 to December 1998 was tested. The median age (range) of women was 27 (13–60) years, of heterosexual men 31 (16–71) years and of homosexual men 31 (18–60) years.

The overall seroprevalence of HSV-1 in 1993 versus 1998 was 68% (695/1024) versus 59% (384/654) (χ^2-test, $P < 0.001$). Likewise, seroprevalence of HSV-2 was 30% (303/1024) versus 22% (141/654) (χ^2-test, $P < 0.001$).

Factors associated with HSV-1 seropositivity in univariate analysis

In both study groups HSV-1 seropositivity increased strongly with age, non-Dutch ethnic background and lower level of education (Table 1). In 1993, HSV-1 seropositivity was significantly associated with 'living in Rotterdam' and there was some evidence for such an association in 1998 (OR = 1.3, 95% CI : 0.9–1.9). In 1993 'having had passive anal intercourse in the past 6 months' was associated with lower HSV-1 seropositivity (OR = 0.5, 95% CI : 0.3–0.8). This effect was not observed in 1998 (OR = 1.3, 95% CI : 0.8–2.1). 'Having worked as or visits to a commercial sex worker' and 'having had >4 sexual partners in the past 6 months' increased the likelihood of being HSV-1 seropositive in both years. A significant association was also noted with 'having a history of STD'. In both years HSV-1 seropositivity was not associated with HIV seropositivity. However, the corresponding 95% CI were wide (Table 3).

Factors associated with HSV-2 seropositivity in univariate analysis

Table 2 shows the univariate analyses of demographic characteristics and sexual behaviour variables with HSV-2 seropositivity in 1993 and 1998. In both study groups, HSV-2 seropositivity showed a strong association with age, with peak levels among those ⩾35 years. In addition, HSV-2 seropositivity was related to ethnic background in both samples. Prevalence of antibody to HSV-2 increased significantly with decreasing level of education in both periods. In 1993, homo/bisexual orientation was significantly associated with higher seroprevalence of HSV-2; this was not observed in 1998. In both periods HSV-2 infection was more prevalent in females. 'Having had passive anal intercourse in the past 6 months' did not prove to be significantly correlated with HSV-2 seropositivity. However, other sexual behaviour variables were predictors for HSV-2 serostatus in both periods (Table 2). Human immunodeficiency virus serostatus was associated with HSV seropositivity in 1993 but not in 1998. In both years, HSV-1 seropositivity was significantly associated with HSV-2 seropositivity.

To determine if HSV-1 and HSV-2 serostatus differed between 1993 and 1998 a multivariate model which adjusted for possible differences in the composition of both study groups was constructed. After correction for possible confounding factors, the seroprevalence of HSV-1 was significantly lower in 1993 compared with 1998 (OR = 0.6, 95% CI : 0.4–0.8, $P = 0.001$). This was also the case for the seroprevalence of HSV-2 (OR = 0.6, 95% CI : 0.4–0.7, $P = 0.001$) (models not shown).

Factors associated with HSV-1 seropositivity in multivariate analyses

Table 3 presents the results of the stepwise backward logistic regression analyses for HSV-1 seropositivity. In multivariate analyses age and non-Dutch ethnic background were associated with HSV-1 seropositivity in 1993 and 1998. Lower level of education ($P < 0.0001$) and female gender ($P < 0.01$) were independently associated with HSV-1 infection in 1993 only. 'Having had passive anal intercourse in the past 6 months' was without an increased associated risk of HSV-1 infection. In 1993, none of the sexual behaviour variables were associated with an increased likelihood of HSV-1 infection. However, there was evidence for such an association in 1998.

A '*z* test' was used to determine whether the differences between the coefficients of the variables 'having had >4 partners in the past 6 months' and 'having a present diagnosis of STD' changed significantly between 1993 and 1998. A borderline significant *P*-value ($P = 0.06$) was calculated for 'having had >4 partners in the past 6 months'. The coefficients of 'having a present diagnosis of STD' did not change significantly between both years ($P = 0.15$).

Factors associated with HSV-2 seropositivity multivariate analysis

Table 3 presents the results of the stepwise backward logistic regression analyses for HSV-2 seropositivity. In both years, increasing age and ethnic background were predictors of HSV-2 seropositivity in multivariate analyses (all variables, overall *P*-value < 0.01) (Table 3), although those of Turkish, North African and Mediterranean origin had no statistically significant increased likelihood of HSV-2 seropositivity. Lower level of education was correlated with HSV-2 seropositivity in 1993 only ($P = 0.01$). Female gender was associated with an increased likelihood of HSV-2 infection in both years. People involved in commercial sex in 1998 were more likely to be HSV-2 seropositive as were those 'having had >4 partners in the past 6 months' in 1993. A 'history of STD' was strongly correlated with past HSV-2 infection in both years.

Additional tests were performed to investigate whether eliminated variables could be re-introduced in the final logistic

Table 1 Univariate analyses of demographic and sexual behaviour profile correlated with antibody to herpes simplex virus (HSV) type 1 among attendees at a sexually transmitted disease (STD) clinic in Rotterdam, The Netherlands, 1993 versus 1998

	HSV-1 1993				HSV-1 1998			
	Total no.[a]	No. +ive	% +ive	OR[b] (95% CI)	Total no.[a]	No. +ive	% +ive	OR (95% CI)
Age (years)								
⩽24	255	152	59.6	1.0	209	105	50.2	1.0
25–29	216	144	66.7	1.4 (0.9–2.0)	140	75	53.6	1.1 (0.7–1.8)
30–34	185	119	64.3	1.2 (0.8–1.8)	102	63	61.8	1.6 (1.0–2.6)
35–39	123	92	74.8	2.0 (1.2–3.2)	78	53	67.9	2.1 (1.2–3.6)
⩾40	245	188	76.7	2.2 (1.5–3.3)	124	87	70.2	2.3 (1.5–3.7)
Ethnic background								
Dutch	636	369	58.0	1.0	395	195	49.4	1.0
Surinamese/Dutch Antilleans	145	111	76.6	2.4 (1.6–3.6)	124	83	66.9	2.1 (1.4–3.2)
Turkish/North African/Mediterranean	109	101	92.7	9.1 (4.4–19.1)	36	27	75.0	3.1 (1.4–6.7)
Other	134	114	85.1	4.1 (2.5–6.8)	99	79	79.8	4.1 (2.4–6.9)
Education								
Higher education	150	78	52.0	1.0	108	56	51.9	1.0
Secondary education	666	441	66.2	1.8 (1.3–2.6)	310	168	54.2	1.1 (0.7–1.7)
Primary education/No education	207	175	84.5	5.0 (3.1–8.3)	236	160	67.8	2.0 (1.3–3.1)
Residence								
Rural	315	187	59.4	1.0	179	96	53.6	1.0
Rotterdam	693	497	71.7	1.7 (1.3–2.3)	475	288	60.6	1.3 (0.9–1.9)
Sexual preference and gender								
Heterosexual male	538	369	68.6	1.0	307	179	58.3	1.0
Homo/bisexual male	83	54	65.1	0.9 (0.5–1.4)	55	33	60.0	1.1 (0.6–2.0)
Female	403	272	67.5	1.0 (0.7–1.3)	292	172	58.9	1.0 (0.7–1.4)
Passive anal contact[c]								
No	905	628	69.4	1.0	580	337	58.1	1.0
Yes	82	45	54.9	0.5 (0.3–0.8)	74	47	63.5	1.3 (0.8–2.1)
Commercial sex[d]								
No	814	541	66.5	1.0	491	272	55.4	1.0
Yes	210	154	73.3	1.4 (1.0–1.9)	156	105	67.3	1.7 (1.1–2.4)
No. of partners past 6 months								
0–4	807	539	66.8	1.0	356	181	50.8	1.0
⩾5	187	134	71.7	1.3 (0.9–1.8)	47	34	72.3	2.5 (1.3–4.9)
History of STD								
None	843	555	65.8	1.0	463	255	55.1	1.0
1×	82	62	75.6	1.6 (1.0–2.7)	154	104	67.5	1.7 (1.2–2.5)
⩾2×	99	78	78.8	1.9 (1.2–3.2)	37	25	67.6	1.7 (0.8–3.5)
Present diagnosis of STD								
No	715	478	66.9	1.0	518	296	57.1	1.0
Yes	309	217	70.2	1.2 (0.9–1.6)	136	88	64.7	1.4 (0.9–2.0)
HIV[e] serostatus								
Seronegative	1012	684	67.6	1.0	648	379	58.5	1.0
Seropositive	12	11	91.7	5.2 (0.7–40.2)	6	5	83.3	3.5 (0.4–29.4)

[a] Numbers do not always add up to total, as some subjects did not answer all questions.

[b] Odds ratio.

[c] Only applicable for homo/bisexual men and women.

[d] For male: visits to a commercial sex worker during the past 6 months; for female: worked as a commercial sex worker during the past 6 months.

[e] Human immunodeficiency virus.

Table 2 Univariate analyses of demographic and sexual behaviour profile correlated with antibody to herpes simplex virus (HSV) type 2 among attendees at a sexually transmitted disease (STD) clinic in Rotterdam, The Netherlands, 1993 versus 1998

	HSV-2 1993				HSV-2 1998			
	Total no.[a]	No. +ive	% +ive	OR[b] (95% CI)	Total no.[a]	No. +ive	% +ive	OR (95% CI)
Age (years)								
≤24	255	45	18	1.0	209	16	8	1.0
25–29	216	50	23	1.4 (0.9–2.2)	140	35	25	4.0 (2.1–7.6)
30–34	185	50	27	1.7 (1.1–2.7)	102	27	26	4.3 (2.2–8.5)
35–39	123	54	44	3.7 (2.3–5.9)	78	24	31	5.4 (2.7–10.8)
≥40	245	104	42	3.4 (2.3–5.2)	124	39	31	5.5 (2.9–10.4)
Ethnic background								
Dutch	636	167	26	1.0	395	59	15	1.0
Surinamese/Dutch Antilleans	145	54	37	1.7 (1.1–2.4)	124	41	33	2.8 (1.7–4.5)
Turkish/North African/Mediterranean	109	16	15	0.5 (0.3–0.8)	36	6	17	1.1 (0.5–2.9)
Other	134	66	49	2.7 (1.9–4.0)	99	35	35	3.1 (1.9–5.1)
Education								
Higher education	150	28	19	1.0	108	17	16	1.0
Secondary education	666	190	29	1.7 (1.1–2.7)	310	62	20	1.3 (0.7–2.4)
Primary education/No education	207	85	41	3.0 (1.8–5.0)	236	62	26	1.9 (1.1–3.5)
Residence								
Rural	315	89	28	1.0	179	41	23	1.0
Rotterdam	693	207	30	1.1 (0.8–1.5)	475	100	21	0.9 (0.6–1.4)
Sexual preference and gender								
Heterosexual male	538	129	24	1.0	307	55	18	1.0
Homo/bisexual male	83	26	31	1.9 (1.2–3.1)	55	8	15	0.8 (0.3–1.7)
Female	403	148	37	2.4 (1.8–3.2)	292	78	27	1.7 (1.1–2.5)
Passive anal contact[c]								
No	905	268	30	1.0	580	125	22	1.0
Yes	82	28	34	1.2 (0.8–2.0)	74	16	22	1.0 (0.6–1.8)
Commercial sex[d]								
No	814	225	28	1.0	491	84	17	1.0
Yes	210	78	37	1.5 (1.1–2.1)	156	52	33	2.4 (1.6–3.6)
No. of partners past 6 months								
0–4	807	220	27	1.0	356	64	18	1.0
≥5	187	73	39	1.7 (1.2–2.4)	47	13	28	1.7 (0.9–3.5)
History of STD								
None	843	209	25	1.0	463	73	16	1.0
1×	82	35	43	2.3 (1.4–3.6)	154	55	36	3.0 (2.0–4.5)
≥2×	99	59	60	4.5 (2.9–6.9)	37	13	35	2.9 (1.4–5.9)
Present diagnosis of STD								
No	715	198	28	1.0	518	106	20	1.0
Yes	309	105	34	1.3 (1.0–1.8)	136	35	26	1.3 (0.9–2.1)
HIV[e] serostatus								
Seronegative	1012	296	29	1.0	627	134	21	1.0
Seropositive	12	7	58	3.4 (1.1–10.8)	6	2	33	1.8 (0.3–10.2)
HSV-1 serostatus								
Negative	329	75	23	1.0	270	49	18	1.0
Positive	695	228	33	1.7 (1.2–2.2)	384	92	24	1.4 (1.0–2.1)

[a] Numbers do not always add up to total, as some subjects did not answer all questions.

[b] Odds ratio.

[c] Only applicable for homo/bisexual men and women.

[d] For male: visits to a commercial sex worker during the past 6 months; for female: worked as a commercial sex worker during the past 6 months.

[e] Human immunodeficiency virus.

Table 3 Multiple logistic analyses for significant variables with specific antibodies to herpes simplex virus (HSV) type 1 and type 2 in 1993 compared to 1998

	HSV-1				**HSV-2**			
	1993		1998		1993		1998	
	OR[a] (95% CI)	Overall *P*-value	OR (95% CI)	Overall *P*-value	OR (95% CI)	Overall *P*-value	OR (95% CI)	Overall *P*-value
Age (years)		<0.0001		0.04		<0.0001		<0.0001
≤24	1.0		1.0		1.0		1.0	
25–29	2.0 (1.3–3.1)		0.8 (0.5–1.4)		2.1 (1.3–3.5)		6.8 (2.7–17.6)	
30–34	1.9 (1.2–3.1)		1.6 (0.8–2.9)		2.4 (1.4–4.1)		6.9 (2.6–18.6)	
35–39	2.9 (1.7–5.0)		1.7 (0.8–3.6)		4.7 (2.7–8.3)		5.4 (1.8–16.9)	
≥40	3.9 (2.4–6.2)		2.2 (1.1–4.3)		6.2 (3.7–10.3)		17.8 (6.1–52.0)	
Ethnic background		<0.0001		0.01		<0.0001		0.01
Dutch	1.0		1.0		1.0		1.0	
Surinamese/Dutch Antilleans	2.2 (1.4–3.4)		1.3 (0.7–2.4)		1.9 (1.2–3.0)		3.5 (1.6–7.9)	
Turkish/North African/ Mediterranean	10.3 (4.7–22.3)		2.2 (0.8–6.2)		0.6 (0.3–1.1)		1.7 (0.4–7.3)	
Other	4.2 (2.5–7.2)		2.9 (1.5–5.8)		3.5 (2.2–5.4)		2.2 (1.0–4.8)	
Education		<0.0001		0.19		0.01		0.82
Higher education	1.0		1.0		1.0		1.0	
Secondary education	2.3 (1.5–3.4)		1.0 (0.6–1.9)		1.9 (1.1–3.1)		1.0 (0.4–2.3)	
Primary education/ No education	4.3 (2.4–7.5)		1.6 (0.8–2.9)		2.4 (1.4–4.3)		1.2 (0.5–2.8)	
Sexual preference by gender and having had passive anal intercourse		0.005		0.84		<0.0001		<0.0001
Heterosexual male	1.0		1.0		1.0		1.0	
Homo/bisexual no passive anal intercourse	2.0 (0.9–4.4)		0.9 (0.4–2.2)		1.2 (0.6–2.2)		1.8 (0.4–8.4)	
Homo/bisexual with passive anal intercourse	0.7 (0.4–1.4)		1.7 (0.3–11.2)		ND[b]		ND[b]	
Female no passive anal intercourse	1.7 (1.2–2.3)		1.0 (0.6–1.7)		3.9 (2.7–5.7)		8.1 (3.4–19.7)	
Female with passive anal intercourse	0.7 (0.3–1.4)		1.5 (0.7–3.1)		2.7 (1.2–6.0)		6.5 (2.1–20.2)	
Commercial sex[c]		NS[d]		NS		0.16		0.0001
No					1.0		1.0	
Yes					1.4 (0.9–2.2)		5.4 (2.3–12.9)	
No. of partners past 6 months		0.91		0.04		0.05		0.32
0–4	1.0		1.0		1.0		1.0	
≥5	1.0 (0.7–1.5)		2.1 (1.0–4.4)		1.5 (1.0–2.5)		0.6 (0.2–1.7)	
History of STD[e]		NS		NS		<0.0001		0.008
None					1.0		1.0	
1×					2.3 (1.3–3.9)		2.7 (1.4–5.3)	
≥2×					3.8 (2.3–6.3)		3.0 (0.7–12.1)	
Present diagnosis of STD		0.45		0.04		NS		NS
No	1.0		1.0					
Yes	1.1 (0.8–1.6)		1.9 (1.0–3.4)					

[a] Odds ratio.

[b] Not determined due to small numbers, cases enclosed in group homo/bisexual male no passive anal intercourse.

[c] For male: visits to a commercial sex worker during the past 6 months; for female: worked as a commercial sex worker during the past 6 months.

[d] Not significant.

[e] Sexually transmitted disease.

Hosmer and Lemeshow goodness-of-fit test for female HSV-1, 1993 model = 6.1, degrees of freedom = 8, significance = 0.63.

Hosmer and Lemeshow goodness-of-fit test for female HSV-1, 1998 model = 10.2, degrees of freedom = 8, significance = 0.25.

Hosmer and Lemeshow goodness-of-fit test for female HSV-2, 1993 model = 8.8, degrees of freedom = 8, significance = 0.36.

Hosmer and Lemeshow goodness-of-fit test for female HSV-2, 1998 model = 8.8, degrees of freedom = 8, significance = 0.36.

Table 4 Crude odds ratio (OR) versus adjusted odds ratio for exposure of herpes simplex virus (HSV) type 1 to herpes simplex virus type 2

	HSV-2 seropositivity			
	Crude OR (95% CI)	*P*-value	Adjusted OR[a] (95% CI)	*P*-value
1993				
HSV-1 seronegative	1.0		1.0	
HSV-1 seropositive	1.8 (1.3–2.5)	0.0003	1.1 (0.8–1.7)	0.50
1998				
HSV-1 seronegative	1.0		1.0	
HSV-1 seropositive	1.5 (0.9–2.5)	0.12	1.0 (0.5–1.8)	0.94

[a] Adjusted for variables age, ethnic background, education, residence, sexual preference by gender and having had passive anal intercourse, worked as or visits to a commercial sex worker, number of partners, history of sexually transmitted diseases (STD), present diagnosis of STD.

regression models for HSV-1 and HSV-2 in Table 3. None of the variables tested, either separately or together, had any significant effect (data not shown).

Exposure-disease model

The crude OR and the adjusted OR from the exposure-disease model describing the effect of HSV-1 seropositivity on susceptibility to genital HSV-2 infection are presented in Table 4. There is strong evidence that HSV-1 seropositivity is associated with HSV-2 seropositivity in 1993 (OR = 1.8, 95% CI : 1.3–2.5) and some evidence for such an association in 1998 (OR = 1.5, 95% CI : 0.9–2.5).

In the final model none of the interaction terms between the exposure variable (HSV-1) and the main effect variables proved to be statistically significant and they were therefore removed by the stepwise backward elimination procedure. In the final model HSV-1 seropositivity was not associated with HSV-2 seropositivity (1993: OR = 1.1, 95% CI : 0.8–1.7; 1998: OR = 1.0, 95% CI : 0.5–1.8).

Discussion

In this study, the seroprevalence of HSV-1 and HSV-2 was examined in a randomly selected group of STD clinic attendees. The independent risk factors correlated with HSV-1 and HSV-2 seropositivity were summarized.

Seroprevalence of HSV-1 was 68% in 1993 compared to 59% in 1998. For HSV-2, seroprevalences were 30% and 22%, respectively. After adjusting for confounding factors in multivariate models these differences remained highly significant (both $P = 0.001$). This supports the conclusion that HSV-1 and HSV-2 infections were becoming less common in our study population comparing 1993 and 1998.

This is the first study to report HSV-1 seroprevalence in The Netherlands and can therefore be used as baseline prevalence for STD clinic populations. The decrease in HSV-1 seroprevalence was most striking in individuals younger than 25 years of age (60% in 1993 versus 50% in 1998). This corroborates observations by others who have reported a decreased incidence of childhood oral HSV infection in Europe.[18,19]

Risk factors for HSV-1 seropositivity in The Netherlands have not been published. In 1993 and 1998 age and ethnic background were correlated with HSV-1 seropositivity. Lower level of education and female gender were only associated with HSV-1 seropositivity in 1993. These risk factors have been reported earlier as being associated with HSV-1 seropositivity in other countries.[19,20]

Several studies have indicated an increasing incidence of genital herpes caused by HSV-1 in the past decade.[11,21,22] This trend has been attributed to 'changes in sexual practices' and falling rates of HSV-1 labial herpes infections in childhood. In this study, none of the sexual behaviour variables were correlated with HSV-1 seropositivity in 1993. However, in 1998 'having had >4 sexual partners in the past 6 months' and 'having a present diagnosis of STD' were linked with HSV-1 seropositivity. These changes in risk factors support the view that sexual transmission of HSV-1 is increasing in The Netherlands.

Since the late 1970s a rise in HSV-2 seroprevalence has been observed in the general population in the US, reaching 22% for the period 1988 to 1994.[3] This increasing prevalence has also been observed in both developed and developing countries.[20,23] Data on HSV-2 seroprevalence in The Netherlands to date are limited to STD clinic attendees. In 1986–1988, HSV-2 seroprevalence was 32% in Amsterdam and 22% in 1998, in Groningen.[9,10] These differences may either indicate that variation of HSV-2 seroprevalence in The Netherlands has a geographical pattern, or that HSV-2 infection is decreasing. The study presented here supports the latter view. In making such comparisons one has to be aware that the selected groups do not represent the general population. However, the falling rate of HSV-2 infection among a population at high risk of STD does not suggest an increasing number of HSV-2 seropositive individuals in the general population. Since HSV-2 seroprevalence can be used as a surrogate marker for sexual lifestyle,[24] this downward trend—mainly in individuals younger than 25 years of age—could be an effect of ongoing national public health campaigns promoting safer sexual practices. Future studies of STD clinic attendees and in the general population may establish whether HSV-2 seroprevalence continues to decrease in The Netherlands.

Risk factors independently correlated with HSV-2 seropositivity were also determined. In 1993 and 1998 these were age, ethnic background, female gender and a history of STD. 'Lower level of education' and 'number of partners in the past 6 months' increased the likelihood of being seropositive for HSV-2 in 1993. 'Having worked as or visits to a commercial sex worker' had a similar effect in 1998. The risk factors reported here confirm the reported recognized risk factors for HSV-2 seropositivity.[3,4,24]

An important hypothesis in HSV research is that prior labial HSV-1 infection reduces the risk of genital HSV-2 infection and has an attenuating effect on the severity of HSV-2.[25–28] This view has been supported by the presence of cross-reactive neutralizing antibodies after HSV infection. In addition, local (cross-reactive) T cell-mediated immune responses have been shown to be important in resolving genital HSV infection.[29] Nonetheless, epidemiological evidence to back up the proposition of reduced susceptibility to HSV-2 infection after an earlier labial HSV-1 infection is based on only a few small case-control studies.[30,31] A large epidemiological study in which the relationship between the presence of HSV-1 and HSV-2 specific antibodies is described was published in 1999.[32] In that prospective study 2393 sexually active HSV-2 seronegative individuals were examined for clinical and serological evidence of new HSV infection. Of the participants, 1508 were seropositive for HSV-1 and 885 were seronegative. It appeared that previous HSV-1 infection did not reduce the incidence of HSV-2 infection. The multivariate exposure-disease model presented in our study provides additional evidence that the risk of genital HSV-2 infection is independent of a previous HSV-1 infection. These results allow the conclusion that HSV-1 antibodies fail to protect against subsequent HSV-2 infection. However, because this conclusion contradicts one of the paradigms in HSV research, additional immunological studies are recommended to corroborate the findings reported here.

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KEY MESSAGES

- HSV-1 and HSV-2 infections are becoming less common among STD clinic attendees in Rotterdam.
- A changing pattern of risk factors correlated with HSV-1 seropositivity indicates that sexual transmission of HSV-1 is increasing.
- A prior HSV-1 infection does not reduce suseptibility to subsequent genital HSV-2 infection.

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Commentary: Developing preventive strategies in Europe

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With the recent increase in availability of relatively cheap and sensitive type-specific antibody tests for Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), the sero-epidemiology of these infections in different countries around the world is becoming better defined. Studies demonstrate wide variation in HSV seroprevalence according to geographical location, socioeconomic and behavioural factors.[1] This study from the University Hospital in Rotterdam, Holland is interesting in that it explores the prevalence of infection among attendees at the same sexually transmitted disease (STD) clinic at two different time points. The authors were able to demonstrate a fall in prevalence of antibodies to both HSV-1 and HSV-2 between 1993 and 1998 of around 40%, after controlling for demographic and behavioural factors. While it is true that this apparent fall in seroprevalence may reflect changes in the clientele attending this STD clinic that have been inadequately controlled for in the statistical analysis, it more likely reflects real changes in the seroprevalence of these infections in this population over this time period.

Although infection with HSV-1 is almost universal in developing countries, more recently prevalence rates as low as 40–50% have been reported among middle-class adults in Western communities. This reduction in prevalence has been attributed to the improvement in living standards in the West over the course of the last century. As in this study from Holland, workers from the Public Health Laboratory Service in the UK have been able to demonstrate that the overall prevalence of antibodies to HSV-1 in the general population has fallen in recent years. In the UK at least, prevalence of antibody to HSV-1 increases with age until about 5 years, then stabilizes until around 15 years old, suggesting that, as has been shown in the Dutch study, sexual transmission is becoming increasingly important as a route of transmission for this virus.[2] HSV-1 is now the commonest cause of primary genital herpes presenting to STD clinics in many areas of the UK.

There has been considerable concern, following publication of HSV-2 seroprevalence data collected as part of the Third National Health and Nutrition Survey (NHANES 3) in the US, that the world is in the grip of an HSV-2 epidemic. NHANES 3 was conducted among a representative sample of adult Americans between 1988 and 1992, and showed that the seroprevalence of HSV-2 among adults in the general population had increased from 16.8% in 1978 (when NHANES 2 was conducted) to 21.7% in 1990.[3] Data from developing countries also demonstrate high levels of infection among certain sectors of the population.[4,5] Widespread population screening of HSV-2 antibody,

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coupled with education aimed at increasing recognition of genital herpes infection has been advocated to control continued spread of infection. However, prevalence rates of antibody to HSV-2 are much higher in the US than in Europe, where there has been much debate about the likely cost-effectiveness of this approach to improve public health.[6] It is therefore interesting that the authors of this Dutch study have demonstrated a fall in the prevalence of HSV-2 antibody among STD clinic attendees between 1993 and 1998, in the absence of HSV-2 antibody screening and specific HSV educational programmes even among STD clinic attendees. It would be nice to think that this reflected a trend towards safer sexual practices among young Dutch people as a result of general education campaigns, although data from other sources are required to substantiate this.

This paper lends further credence to the view that strategies for managing genital herpes at a population level need to be tailored to the local population and that European countries should not be pressured into such strategies on the basis of evidence accrued in the US alone. We need to get more evidence about the seroprevalence of HSV-1 and HSV-2 in both the general, STD and antenatal clinic populations across Europe in order to make informed decisions about likely necessity of introducing screening in these different settings. Formal evaluation of the likely costs and benefits of screening need to be established in different settings to ensure it will indeed benefit the public health.

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