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CLINICAL REPORT

Importance of Herpes Simplex Virus Type-1 (HSV-1) in Primary Genital Herpes

Rutger F. NIEUWENHUIS[1], Gerard J. J. van DOORNUM[2], Paul G. H. MULDER[3], H. A. Martino NEUMANN[1] and Willem I. van der MEIJDEN[1]

Departments of [1]Dermatology and Venereology, [2]Virology and [3]Epidemiology and Biostatistics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Herpes simplex virus type 1 (HSV-1) is increasingly reported in primary genital herpes. Its incidence was assessed among Rotterdam sexually transmitted diseases clinic attendees between 1996 and 2001, and demographic and sexual behaviour factors were evaluated. A retrospective record analysis was performed. All herpes diagnoses were based on cell culture techniques. A clinical scoring system was used to select "primary" cases. Demographic and sexual behaviour characteristics were analysed using logistic regression. The clinical scoring system showed 115 cases of primary genital herpes. HSV-1 (n=60) was found in 52% and HSV-2 (n=55) in 48% of cases. The multiple logistic regression model showed that HSV-1 was associated with "oro-genital contact" (p<0.001) and "having a single partner in the last 2 months" (p=0.054) and that HSV-2 was associated with "a higher number of sexual partners in the last 6 months" (p=0.085). Our data confirm the growing importance of HSV-1 in primary genital herpes; oro-genital sex is the main risk factor. ***Key words: primary genital herpes; HSV-1; epidemiology; risk factors.***

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Willem I. van der Meijden, Erasmus MC, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. E-mail: w.i.vandermeijden@erasmusmc.nl

Genital herpes is an ulcerative disease of the ano-genital skin and mucosa, and is characterized by latency and intermittent clinical and subclinical reactivity and infectiousness (1). The prevalence of genital herpes is growing in both the western world and developing countries (2–4) and is the leading cause of genital ulcer disease (GUD) in the western world (5). This is worrisome since herpes simplex virus (HSV) infection may enhance the sexual transmission of the human immunodeficiency virus (HIV) (6), and may even accelerate the onset of acquired immunodeficiency syndrome (AIDS) (7). Moreover, both clinical and subclinical reactivations of HSV are more common in immunocompromised patients. This synergy between HSV and HIV calls for continued public awareness.

Data on the prevalence of genital herpes is based mainly on national HSV type 2 (HSV-2) seroprevalence surveys (3, 8, 9). However, a growing number of reports show that HSV type 1 (HSV-1) is increasingly associated with primary genital herpes in the western world (10–12). Therefore, the prevalence of genital herpes may be seriously underestimated. The HSV-2 seroprevalence among sexually transmitted disease (STD) clinic attendees in Amsterdam was 32.3% in the late 1980s, which has long served as the baseline prevalence for STD clinic populations in The Netherlands (9).

Between 1993 and 1998, the Rotterdam STD clinic population showed a decrease by almost 10% in the seroprevalence of both HSV-2 and HSV-1 (13). However, the number of genital herpes cases registered in the Dutch STD surveillance system has increased sharply in recent years (14). We hypothesized that HSV-1 has become the major cause of primary genital herpes among patients visiting the Rotterdam STD clinic. We studied attendees with genital herpes in the period 1996 to 2001, and analysed demographic and sexual behaviour characteristics.

MATERIAL AND METHODS

Study population and data collection

Patients attending the outpatient clinic for STDs at the Erasmus MC in Rotterdam between January 1996 and December 2001, and having a positive HSV culture from ano-genital specimens, were studied retrospectively. Laboratory data were obtained from the automated system of the Department of Virology.

All data were collected from the patients' records, and were registered in a database using SPSS 10.0 software. Medical history, and location, extent and character (vesicles, erosions and/or ulcerations) of skin lesions were recorded. Standardized questionnaires have been used for a long time at the STD clinic, covering both demographic and sexual behaviour characteristics. All patients underwent a routine STD examination, as described by Van der Snoek et al. (15). In our clinic, serum specimens to perform IgM and IgG assays were only obtained in patients who, on the basis of the clinical appearance of their skin lesions, were suspected of having primary genital herpes. In these cases, patients were asked to visit the clinic after 2 weeks for a second serum specimen.

Clinical specimens and culture technique

All herpes diagnoses were based on cell culture techniques, as described elsewhere (16). Briefly, ano-genital specimens were obtained using cotton-wool-tipped swabs. Specimens were transported to the laboratory in a virus transport medium,

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consisting of minimal essential medium-HEPES balanced salt solution (BioWhittaker, Verviers, Belgium) supplemented with 10% foetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml). Monolayers of human embryonic lung fibroblast cells were cultured in 24-well microtitre plates; 200 µl of the specimen was inoculated in duplicate and centrifuged at 3500 × g for 15 min at room temperature. After 48 h, one part of the cells was incubated with monoclonal antibodies against HSV-1 or HSV-2 (De Beer Medicals, Uden, The Netherlands, and Imagen, Dako Diagnostics, Cambridgeshire, UK), and when indicated also with monoclonal antibodies against VZV or CMV (Argene-Biosoft, Varilhes, France), separately. The other part of the cell culture was maintained for 14 days. When a cytopathic effect was observed, cells were incubated again with the monoclonal antibody specific for the typical cytopathic effect.

Serological techniques

Presence of HSV-1 and HSV-2 antibodies was determined using the COBAS Core Anti-HSV-I/II enzyme immunoassay (EIA) (Roche Diagnostic GmbH, Mannheim, Germany). This is an indirect two-step EIA for the qualitative detection of human antibodies to herpes simplex viruses. The COBAS Core HSV-2 IgG EIA (Roche Diagnostics GmbH, Mannheim, Germany) was used to establish the presence of specific IgG against HSV-2. This also is an indirect two-step EIA. Detection of HSV IgM was performed using the HSV IgM EIA (Meddens Diagnostics BV, Vorden, The Netherlands). This is an IgM antibody-capture ELISA for the detection of HSV specific IgM in human serum based on whole HSV-2 virus antigen.

Substudy on diagnosing "primary" genital herpes

Serology was regarded as the gold standard to determine whether a genital HSV infection was new or pre-existing. However, as mentioned above, these tests were not routinely performed in our clinic. The serological diagnosis of "primary" genital herpes was defined by the absence of IgG antibodies, or high IgM antibodies combined with low IgG antibodies in the first serum specimen, or an increase in the IgM antibody titre.

In daily practice, the diagnosis of "primary" genital herpes is usually based on the clinical presentation without the use of serology. We refer to this as the "diagnosis at a glance". In this study, we diagnosed primary cases by the use of a clinical scoring system (CSS), based on patients' clinical features and history (Table I). The CSS can be described as the weighted sum of clinical characteristics, with the weights indicating the clinical importance of each characteristic. The weight was based on the log odds ratio of the characteristic in a multiple logistic regression model. HSV serology, performed in 73 patients, was used as the reference method for validation of the scoring system.

Statistical analysis

To test if the CSS could better predict primary disease than "diagnosis at a glance", both were simultaneously introduced in a logistic regression model, using the likelihood-ratio (LR) test, with serological outcome as dependent variable. In addition, the sensitivity and specificity with 95% confidence interval (95% CI) were calculated for both methods, with serology as the reference.

Univariable analyses were performed to investigate the relationship between demographic or sexual behaviour characteristics and the type of HSV infection in a group of patients with primary genital herpes. The χ^2 test was used for the categorical variables and the *t*-test (or Mann-Whitney test when appropriate) for the continuous variables. Variables with a *p*-value below 0.20 were selected to enter simultaneously as independent variables in a multiple logistic regression analysis, with type of HSV-infection as dependent variable. The final multiple logistic regression model was obtained after stepwise backward elimination of the independent variables using the LR-test with $p < 0.10$. Finally, all variables considered and not included in the multiple logistic regression analysis were re-entered one-by-one in the final model, in order to confirm the selection. The strength of the relationship between independent variables and type of HSV-infection was expressed by means of an odds ratio (OR) with 95% CI and *p*-value.

Table I. *Clinical scoring system (CSS) used to assess primary genital herpes*[a]. *A primary infection is defined by score ≥5.*

Characteristics	Weighted CSS score
I. No history of genital herpes	1
II. Painful ano-genital skin lesions	3
IIIa. Few, but bilateral ano-genital skin lesions (mild eruption)	2[a]
or	
IIIb. Numerous ano-genital skin lesions (severe eruption)	3[a]
IV. Skin lesions are not grouped	2
V. Tender lymphadenopathy	1

[a]The maximum score adds up to 9 (IIIa) or 10 (IIIb)

RESULTS

Study population

A total of 534 patients (270 males and 264 females, mean age ± SD 32.6 ± 11.4, range 12–82 years) presented with genital herpes between 1996 and 2001. HSV-2 was found in the majority of these patients (*n*=403; 75%). Proportionately, HSV-2 was more common in males than in females. The ratio of HSV-1 to HSV-2 was 1:5 in males, compared with 1:2 in females.

HSV serology using paired serum specimens was performed in 73 patients with suspected primary genital herpes. This showed 49 (68%) "primary" and 24 (32%) "recurrent" cases. A score of 5 or more in the CSS was found in 35 of 49 "primary" cases (71%) and 5 of 24 "recurrent" cases (21%).

Using the CSS in all 534 genital herpes patients, 115 patients (22%) were considered to have a "primary" infection. This group consisted of 79 females and 36 males (28 heterosexual, 7 homosexual, 1 bisexual). All patients were sexually active. The mean age ± SD in this group was 26.4 ± 8.1 years (range 12–54 years). HSV-1 was slightly more predominant than HSV-2 (*n*=60; 52%), and there was no difference in the HSV-1 to HSV-2 ratio between males and females (data not shown). Fig. 1 illustrates the HSV-1 and HSV-2 proportions per year of both the "primary" and "recurrent" genital herpes group.

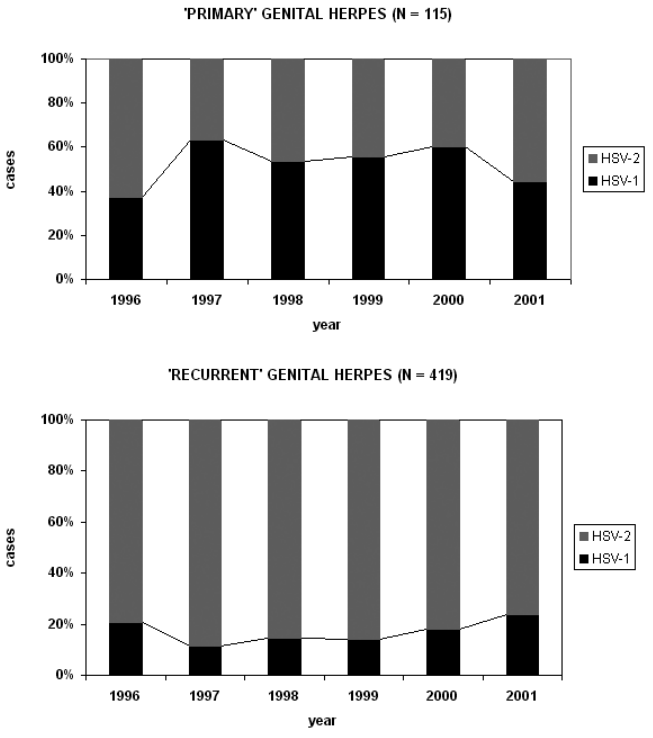

Fig. 1. Proportions of HSV-1 and HSV-2 in primary genital herpes (n=115) and recurrent genital herpes (n=419) per year at the Rotterdam STD clinic in the period 1996 to 2001.

Substudy on diagnostic performance of the CSS and "diagnosis at a glance"

The CSS had a better diagnostic performance than "diagnosis at a glance". The sensitivity of the CSS was 0.71 (95% CI 0.57–0.83) and the specificity was 0.79 (95% CI 0.58–0.93). The "diagnosis at a glance" had a sensitivity and specificity of 0.63 (95% CI: 0.48–0.77) and 0.71 (95% CI 0.49– 0.87), respectively. Moreover, additional logistic regression analysis showed that the CSS was a better predictor of serology. When simultaneously introduced in a logistic regression model, the CSS adjusted for "diagnosis at a glance", could significantly better predict results of serological examination (p = 0.002) than "diagnosis at a glance" adjusted for CSS (p = 0.538).

Main study on demographic and sexual behaviour characteristics

Table II shows the results of univariable and multivariable analyses in the 115 patients who were, on the basis of the CSS, diagnosed with "primary" genital herpes. Because of our interest in the determinants of "primary" genital herpes, the statistics were only performed in this group of patients (n = 115). The variables "educational level", "condom use", "HIV infection" and "history of herpes labialis" were excluded from the analyses, because of too many missing data. Variables included and with a p-value lower than 0.2 in the univariable analysis were entered simultaneously in a logistic regression model. The final multiple logistic regression model resulted from stepwise backward elimination on the basis of the likelihood-ratio test, with the remaining variables significantly associated with the type of genital HSV infection adjusted for each other. The variables in the multiple logistic regression model were "number of sexual partners in the last 6 months" (p=0.085), "a single partner for at least 2 months" (p=0.054) and "oro-genital sex in the last 6 months" (p<0.001). All other variables were not significant after re-entering one-by-one in the final model: the lowest p-value was 0.12. This finding confirmed that the selected variables were simultaneously related to genital herpes. The final logistic regression model is shown in Table III. In this model, the presence of variables in favour of either HSV-1 or HSV-2 depends on the value of the OR. An OR < 1 denotes an effect in favour of HSV-1 and an OR > 1 denotes an effect in favour of HSV-2. The results show that the variables "oro-genital sex in the last 6 months" and "a single partner for at least 2 months" were associated with HSV-1. "A higher number of sexual partners in the last 6 months" was associated with HSV-2.

DISCUSSION

In order to assess the prevalence of HSV-1 in patients suspected of having "primary" genital herpes, and presenting at the Rotterdam STD clinic, a retrospective analysis of clinical and laboratory data covering a 6-year period was performed. In addition, demographic and sexual behaviour characteristics of these patients were evaluated. Our data showed that "primary" genital herpes was caused by HSV-1 in a small majority (52%), which is in accordance with findings from studies outside The Netherlands (10–12).

Primary genital herpes is a genital HSV infection in an individual not earlier exposed to HSV. A typical case of primary genital herpes is clinically characterized by an extensive skin eruption consisting of multiple (mostly bilateral) vesicles and/or erosions (or ulcers) in the anogenital region. It is often accompanied by tender inguinal lymphadenopathy and sometimes fever. A recurrence is usually characterized by a milder and shorter clinical course due to previously acquired serum antibodies to the homologous type of HSV (17). A first clinical episode of genital herpes in an individual with previously acquired heterologous HSV antibodies (usually HSV-1 from herpes labialis in childhood) is referred to as "non-primary first episode" (NPFE) (17, 18). NPFE can only reliably be diagnosed by the use of type-specific serology, which was not available in this study. Therefore, we did not consider NPFE in this study.

Table II. *Results of univariable and multivariable analysis of demographic and sexual behaviour factors in a group of patients with "primary" genital herpes, n = 115*[a]

Variable	HSV-1	*HSV-2*	Univariable analysis	Multivariable analysis[b]
Year of first episode[c] *(n)*			0.74	0.74
1996	6	10		
1997	11	8		
1998	8	7		
1999	15	12		
2000	12	8		
2001	8	10		
Gender[c] *(n)*			0.55	0.35
Male	17	19		
Female	43	36		
Age[e], median (range) (years)	26 (12-54)	24 (17-50)	0.36	0.58
Ethnic background (n)			0.13[f]	0.31
The Netherlands	39	27		
Surinam/Dutch Antilles	5	14		
Other	9	7		
Sexual preference[c] *(n)*			0.85	1.00
Heterosexual	56	50		
Homosexual	4	3		
Bisexual	0	1		
Age of first sexual contact[d] (median (range) (years)	17 (12-29)	17 (12-30)	0.58	0.96
Single sexual partner for at least 2 months[c] *(n)*			0.004[f]	0.054[g]
Yes	51	36		
No	5	17		
No. of partners in last 6 months[d] (median (range))	1 (0-4)	1 (1-15)	0.025[f]	0.085[g]
N*o. of life-time sexual partners*[e] (median (range))	2 (0-100)	5 (1-100)	0.003[f]	0.12
Oro-genital contacts in last 6 months[c] *(n)*			< 0.001[f]	< 0.001[g]
Yes	45	24		
No	8	23		
Anal sex in last 6 months[c] *(n)*			0.78	0.25
Yes	7	7		
No	48	40		
History of STD[c] *(n)*			0.043[f]	0.23
Yes	7	16		
No	49	38		
Concomitant STD[c] *(n)*			0.36	0.98
Yes	10	15		
No	35	33		

[a]The sum does not always add up to total due to missing data. [b]*p*-values of characteristics not marked [g] refer to re-entry in the final logistic model. [c]χ^2-test. [d]*t*-test. [e]Mann-Whitney test. [f]Candidates for inclusion in the multiple logistic regression model ($p < 0.2$). [g]*p*-values of variables simultaneously included in the final multiple logistic regression model.

Type-specific HSV serology is the "gold standard" to diagnose a primary infection with HSV (18). However, this technique is relatively expensive, time-consuming and not widely available for commercial use (19). Diagnosing "primary" cases on the basis of clinical manifestations without the use of serology is cumbersome, because of the wide spectrum of clinical appearances. Even experienced clinicians can easily misinterpret the clinical picture. Diamond et al. (20) reported that about 10% of patients with serological evidence of reactivation showed a clinical picture similar to that of a primary infection. Thus, a "diagnosis at a glance" may lead to misinterpretation of the clinical appearance, especially in atypical cases.

Because of the limited number of serological assays (n=73) in the study period, we designed a CSS. It was formulated to meet the most objective measure for clinical judgement of the herpes lesions, which enabled us to diagnose "primary" cases more accurately compared with "diagnosis at a glance". All our "primary" diagnoses were based on the CSS. It can be regarded as a clinical algorithm to improve the reliability and objectivity to discriminate between "primary" and "recurrent" disease in this study. However, a drawback of using this tool is the rather low sensitivity (0.79) and specificity (0.71). Therefore, type-specific serology remains the recommended method for those patients who are anxious to know the nature of infection.

In the overall genital herpes study group (n=534), HSV-2 was predominantly (75%) isolated. Considering only the group of patients with – on the basis of the CSS – "primary" genital herpes (n=115), the HSV-2 proportion was only 48%. It seems plausible that the majority of HSV-1 infections in primary genital herpes are caused by the oro-genital route of transmission. Moreover, subclinical shedding of HSV-1 from the oral

Table III. *Final multiple logistic regression model with the probability of variables to be independently associated with primary genital HSV-1 or HSV-2 infection*

Independent risk factor	OR (95% CI)[a]	Associated with:	p-value
Oro-genital sexual contact in the last 6 months	0.18 (0.06–0.49)	HSV-1	< 0.001
Single sexual partner for at least 2 months	0.30 (0.09–1.06)	HSV-1	0.054
Higher number of sexual partners in the last 6 months	1.61 (0.84–3.08)	HSV-2	0.085

[a]OR < 1 is effect in favour of HSV-1; OR > 1 is effect in favour of HSV-2
OR, odds ratio; CI, confidence interval.

cavity has been well-documented (21). Multivariable analysis of demographic and sexual behaviour characteristics confirmed that "oro-genital sex in the last 6 months" was by far the most important risk factor for HSV-1 as the cause of primary genital herpes ($p<0.001$; OR=0.18). Another independent predictor for primary genital HSV-1 infection was "having a single partner" ($p=0.054$; OR = 0.30). In contrast, "a higher number of sexual partners in the last 6 months" seemed to be more often associated with primary genital HSV-2 infection ($p=0.085$; OR = 1.61).

Although the age of sexual debut varies significantly worldwide (22), there is a tendency towards a lower age in most countries (23). In case of earlier genital HSV-2 acquisition, subsequent HSV-2 antibodies may, to a certain degree, protect against genital HSV-1 infection. However, a younger age at first sexual contact was previously found to be an important predictor of genital HSV-1 infection (24). In our study, age at first sexual contact in patients with "primary" genital HSV-1 did not differ from that in patients with "primary" genital HSV-2 infection ($p=0.58$), neither was there a significant difference in the age at which genital HSV-1 and HSV-2 infection were acquired ($p=0.96$).

Ethnicity has been associated with the type of HSV infection (25, 26). It was found that HSV-1 is significantly less frequently isolated from genital lesions in patients with an ethnic background of lower socio-economic status. Although HSV-2 was the main cause of clinical genital herpes in these populations, the HSV-1 seroprevalence in these populations was high (25). This suggests that circulating HSV-1 antibodies due to herpes labialis, usually acquired before becoming sexually active, protect against genital HSV-1 infection in the sexually active life-phase (2).

Due to Dutch colonial history, the Rotterdam region harbours a large Surinamese/Dutch Antillean community. Therefore, we examined three ethnic groups in this study, i.e. "Dutch", "Surinamese/Dutch Antillean" and "other" (e.g. African, South- American, East-European, Asian). Among the Surinamese/Dutch Antillean patients with primary genital herpes, HSV-1 was found to be less common than HSV-2. On the contrary, Dutch patients more often showed HSV-1 infection. Although multivariable analysis ($p=0.31$) did not reveal a significant difference, these data show a similar trend compared with findings from other studies (25). The high p-value may be explained by the low number ($n=19$) of primary cases in the Surinamese/Dutch Antillean population. In addition, sexual behaviour could not be analysed because of the limited number of Surinamese/Dutch Antillean patients included in the study. Therefore, it cannot be concluded from our data that the different numbers of HSV-1 and HSV-2 isolations in primary genital herpes in the two ethnic groups mentioned above are caused by a different sexual behaviour (e.g. more oro-genital contact in Dutch patients). It seems likely, however, that these differences are based on pre-existing antibodies due to a non-genital HSV infection earlier in life.

Documentation of both the changes in HSV epidemiology and (sexual) behaviour is important to maintain or improve the quality of patient counselling. First, counsellors should point out the chronic character of genital herpes, and the possibility of subsequent HSV transmission to sexual partners, even between clinical episodes (subclinical shedding). Secondly, patients should be aware that oro-genital contact poses an increased risk of HSV-1 transmission to the ano-genital skin. Thirdly, the lower tendency of HSV-1 to reactivate is of prognostic value, since genital herpes is often accompanied by psychological distress (27). Fourthly, the possibilities for treatment, i.e. episodic or prophylactic regimens, can be discussed and explained. And, finally, when a female patient is HSV sero-negative, she needs to be informed about the risk of primary genital herpes occurring in late pregnancy. This may be associated with herpes in the newborn by vertical transmission and can be devastating (28).

In conclusion, HSV-1 was the major cause of primary genital herpes within the Rotterdam STD clinic population during the period 1996 to 2001. The main risk factor for primary HSV-1 infection in the studied population was "oro-genital contact", whereas "a higher number of different sexual partners" increased the risk of primary HSV-2 infection. Type-specific serology may be useful in the management of first-episode genital herpes (18). By our efforts to create a clinical algorithm, we once again learned that, for the time being, type-specific serology is the most reliable tool to differentiate between primary and recurrent herpes. As such, the use of type-specific serology can be instrumental to improve patient counselling.

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