ORIGINAL ARTICLE

Herpes simplex virus type 1 is the leading cause of genital herpes in New Brunswick

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INTRODUCTION: Little is known about the role of herpes simplex virus (HSV) type 1 (HSV1) in the epidemiology of genital herpes in Canada. Data on herpes viral cultures for two consecutive years obtained from L'Hôpital Dr GL Dumont, which performs all the viral culture testing in New Brunswick, were reviewed. It was hypothesized that HSV1 was the main cause of genital herpes in New Brunswick.
METHODS: Samples of genital origin sent to the laboratory for HSV culture testing between July 2006 and June 2008 were analyzed. Samples from an unspecified or a nongenital source were excluded from analysis. Multiple positive samples collected from the same patient were pooled into a single sample.
RESULTS: HSV was isolated from 764 different patients. HSV1 was isolated in 62.6% of patients (male, 55%; female, 63.8%). HSV1 was isolated in 73.2% of patients 10 to 39 years of age and in 32% of patients ≥40 years of age. The difference in rates of HSV1 infection between the 10 to 39 years of age group and the ≥40 years of age group was statistically significant (P<0.001 [χ^2]). In a similar Canadian study performed in Nova Scotia, HSV1 was recovered in 53.7% of positive cultures (male, 36.7%; female, 58.1%). The rates of HSV1 infection reported by this study and the present study were significantly different (P<0.001 [χ^2] for male, P=0.012 for female).
CONCLUSION: In New Brunswick, HSV1 is the dominant type of HSV isolated in samples collected from a genital site. Significant rate differences were demonstrated between the groups 10 to 39 years of age and ≥40 years of age.

L'herpès simplex de type 1 est la principale cause d'herpès génital au Nouveau-Brunswick

INTRODUCTION : On ne sait pas grand-chose du rôle du virus de l'herpès simplex de type 1 (VHS1) dans l'épidémiologie de l'herpès génital au Canada. Les chercheurs ont analysé les données des cultures d'herpès viral obtenues pendant deux années consécutives à L'Hôpital Dr-Georges-L.-Dumont, où toutes les cultures virales sont effectuées au Nouveau-Brunswick. Ils ont postulé que le VHS1 était la principale cause d'herpès génital dans la province.
MÉTHODOLOGIE : Les chercheurs ont analysé les prélèvements génitaux envoyés en laboratoire entre juillet 2006 et juin 2008, en vue de cultures du virus d'herpès simplex (VHS). Ils ont exclu de l'analyse les prélèvements tirés d'une source non précisée ou d'origine non génitale. Ils ont regroupé dans un seul échantillon les multiples échantillons positifs prélevés chez le même patient.
RÉSULTATS : Le VHS a été isolé chez 764 patients, et le VHS1, chez 62,6 % des patients (55 % chez les hommes, et 63,8 % chez les femmes), soit 73,2 % des patients de dix à 39 ans et 32 % des patients de 40 ans et plus. La différence de taux d'infection par le VHS1 entre le groupe des dix à 39 ans et celui des 40 ans et plus était statistiquement significative (χ^2, P<0,001). Dans une étude canadienne similaire menée en Nouvelle-Écosse, 53,7 % des cultures positives contenaient le VHS1 (36,7 % chez les hommes, 58,1 % chez les femmes). Le taux d'infection par le VHS1 déclaré dans cette étude et celui de la présente étude différaient de manière significative (χ^2, P<0,001 chez les hommes, P=0,012 chez les femmes).
CONCLUSION : Au Nouveau-Brunswick, le VHS1 est le principal type de VHS isolé dans des échantillons d'origine génitale. On a établi des différences de taux significatives entre le groupe des dix à 39 ans et celui des 40 ans et plus.

Key Words: *Canada; Genital herpes; Herpes simplex virus type 1*

Herpes simplex virus (HSV) type 2 (HSV2) is the leading cause of genital ulcers worldwide (1), but either HSV type 1 (HSV1) or HSV2 may be the cause (2). The relative role of HSV1 in herpes simplex genital infections varies from 3.9% to 78% (3,4). Most studies have relied on herpes simplex type-specific serology in estimating the significance of HSV2 in causing sexually transmitted infections. However, HSV1 is commonly acquired as an orolabial infection, so antibodies against it cannot be conclusively linked to genital infections. Using methods such as viral culture or molecular assays, it is possible to ascertain the role of HSV1 in causing genital ulcers. In our experience, many samples of genital origin tested in our laboratory grew HSV1. Given that our laboratory has tested all samples from New Brunswick for HSV since 2006 and given the sparsity of Canadian data, we decided to analyze two years of sample results (July 2006 to June 2008), hypothesizing that HSV1 would be the dominant type of HSV identified in those samples.

METHODS

There are no privately operated laboratories in New Brunswick. L'Hôpital Dr GL Dumont, located in southeast New Brunswick, is the only viral testing laboratory in the province, and has provided HSV culture services since 1992. New Brunswick laboratories ceased referring samples for HSV culture outside the province during the spring of 2006, when New Brunswick had a population of 748,000 (5). A review of the number of samples referred from other laboratories before and after the spring of 2006 confirmed that all samples collected for herpes simplex direct testing were referred to the laboratory at L'Hôpital Dr GL Dumont after this period. Molecular testing of HSV on genital samples was not available routinely in New Brunswick at the time of the present study, and HSV type-specific serology is not performed in New Brunswick laboratories.

Samples submitted to the laboratory included the name, age and date of birth of the individual and the anatomical area collected for testing (eg, buttock). Most samples were also submitted with a unique identifying number (Medicare and/or hospital medical record number). It is not known if the samples were collected from a primary episode or a recurrence. Double counting was prevented by reviewing all positive samples. All positive samples collected from the same patient, either at the same or at different times, were pooled and counted as one sample.

Samples were collected with the Starplex Starswab Multitrans Collection and Transport System (Starplex Scientific Inc, Canada) or

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TABLE 1
Distribution of herpes simplex virus (HSV) type 1 (HSV1) and type 2 (HSV2) in samples collected from genital lesions for viral culture testing, according to age group and sex in New Brunswick between July 2006 and June 2008

	Age group, years										
	10–14	15–19	20–24	25–29	30–39	40–49	50–59	60–69	70–79	>80	Total
Women											
HSV1, n	6	99	123	73	74	25	20	5	2	0	427
HSV2, n	0	24	36	33	40	45	34	12	14	4	242
Total, n	6	123	159	106	114	70	54	17	16	4	669
HSV1/HSV1+HSV2, %	100	80.5	77.4	68.9	64.9	35.7	37	29.4	12.5	0	63.8
Men											
HSV1, n	0	8	26	15	13	9	2	1	0	0	74
HSV2, n	0	2	10	3	14	13	11	5	3	0	61
Total	0	10	36	18	27	22	13	6	3	0	135
HSV1/HSV1+HSV2, %	0	80	72.2	83.3	48.1	40.9	15.4	16.7	0	0	54.8

the Copan UTM 3 mL kit (Copan Diagnostics, Inc, USA). Samples were then stored and shipped at −70°C.

Samples submitted in the wrong transport medium, at room temperature or without sufficient identifiers were not processed and were excluded from the study.

The inoculation of processed samples was performed using flat-bottom 96-well microplates, with each sample being inoculated in two wells of each of the following four cell lines: A549, MRC-5, Mink and Vero. The microplates were then centrifuged at 1600 rpm for 60 min at 33°C, after which they were incubated at 35°C in 5% CO_2 for 10 days. The microplates were inspected daily for cytopathic effect (CPE) using an inverted microscope.

The supernatant of wells showing CPE affecting at least 75% of the cells were harvested for subsequent passage in a vial containing the same cell line. Cells harvested from an infected well were deposited on a 10-well slide for staining with type-specific HSV monoclonal immunofluorescent antibodies (MFA) (Thermo Fisher Scientific, USA). Only samples displaying a typical CPE and typed by MFA were reported as positive for HSV1 or HSV2. Samples with CPE typical of a herpes virus that could not be typed as HSV1 or HSV2 were then further tested for cytomegalovirus or varicella zoster virus by MFA. Samples with a typical or an atypical CPE that could not be typed with the regular set of MFA were not retested with a different set of MFA or by a molecular method. Cultures without CPE after 10 days were reported as negative.

Results from all samples submitted for HSV culture from July 2006 to June 2008 were studied. Results from samples that had grown HSV1 or HSV2 were retrieved and inspected by one of the authors (DL). Results from genital site samples that had grown either HSV1 or HSV2 from patients older than nine years of age were included in the present study.

RESULTS

Between July 2006 and June 2008, 4263 sample results were included for analysis. HSV had been isolated in 1304 samples, from which 805 genital samples from 764 patients were positive for HSV. No samples were infected by both HSV1 and HSV2, either in samples collected from a single date or multiple dates. The available data did not allow determination of the number of rejected samples, samples with an atypical CPE or with an isolate that could not be typed. No sample negative for HSV by viral culture was retested by a molecular assay.

HSV1 was identified in 62.6% of all positive patients and was more frequent in females (63.8%) than in males (55%) (Table 1). Among patients with a positive genital sample, female patients were over-represented (635 female patients, 129 male patients), but the difference in the HSV1 discovery rates between male and female patients was not significant. No significant regional differences were seen in the rate of HSV1/HSV2 genital infections.

In comparison, in the ≥40 years of age group, the rate of occurrence of HSV1 dropped significantly. The difference in the rates of occurrence of HSV1 between the combined population of males and females for the age groups 10 to 39 years (77%) and ≥40 years (32%) was statistically significant ($P<0.001$ [χ^2]).

DISCUSSION

In 1992, our laboratory started providing HSV viral culture services, and from our observations we suspected that HSV1 was the dominant strain of HSV from samples of genital origin. Until recently, we were unable to prove this observation; however, since the spring of 2006 we have processed viral cultures from all New Brunswick hospitals and now have gathered enough data to confirm this hypothesis. A detailed review of the number and type of samples received since June 2006 make us confident that we have gathered all samples collected for HSV detection in New Brunswick. Double counting was ruled out because all positive samples collected in duplicate from a single patient were reviewed by one of the authors (RG) and counted as a single sample.

Viral culture was used as a surrogate for incident infection. We were unable to determine if the isolated HSV was from a primary or a recurrent episode. The present study may have underestimated the role of HSV1 in episodes of genital herpes because the median recurrence rate for patients with HSV2 is much greater than that for patients with HSV1 infection (6). On the other hand, there is a marked predominance of HSV1 isolates in patients presenting to their primary care provider with a first, symptomatic genital herpes infection (7). How these two observations affect our results is difficult to estimate.

HSV1 is the main cause of genital herpetic lesions in New Brunswick, for both men and women, and predominantly affects patients <40 years of age. This observation confirms the results of a Canadian study by Foward and Lee (8) concluded eight years ago in Nova Scotia. The age groups differ slightly because the study by Forward and Lee included patients ≥16 years of age; however, the number of patients in our cohort of nine to 15 years of age was negligible (six patients). Women constituted the bulk of the cases in our cohort, accounting for 83.2% of all cases of genital herpes. The cohort of women 15 to 39 years of age was our largest group of patients infected with HSV1 (55.1%). This was also the case in the study by Forward and Lee (79.3%). The lower rate of HSV1 in older patients in our study may represent a lower incidence of HSV1 in this age group due to different sexual practices, but may also reflect the tendancy of HSV1 to reactivate less frequently in comparison with HSV2. We cannot exclude the possibility that patients with recurrent genital herpes may not have presented to their care provider for diagnosis because they were already aware of their condition. This may explain the relatively low number of patients tested for genital herpes in the present study. Older patients infected with genital herpes in the present study may then be primary symptomatic cases, while younger patients may represent recurrent infections.

HSV1 is transmitted through contact with a person who is shedding virus at a peripheral site, a mucosal surface or in genital or oral secretions. Aerosol and fomite spread are unusual means of

TABLE 2
Studies on the role of herpes simplex virus type 1 (HSV1) as a cause of genital herpes infection, based on viral culture or nucleic acid test

Author (reference), year	Period	Country	Population studied	Overall HSV1 proportion, %
Nilsen and Myrnel (19), 2000	1987–1989	Norway	Outpatient clinic	36.0
	1992–1995			66.0
	1996–1998			51.0
Löwhagen et al (20), 2000	1995–1999	Sweden	STD clinic	44.0
Roberts et al (4), 2003	1993–2001	United States	University clinic	1993: 31.0
				2001: 78.0
Bruinsten et al (3), 2001	1996	Netherlands	STD clinic	3.9
Nieuwenhuis et al (21), 2006	1996–2001	Netherlands	STD clinic	52.0
Manavi et al (22), 2004	1998–2001	Scotland	STD clinic	54.3
Nilsen et al (23), 2007	1999–2001	Tanzania	STD clinic	8.6
Theng and Chan (24), 2004	2001	Singapore	STD clinic	11.6
Zmira et al (25), 2003	1993–2002	Israel	Hospital laboratory	66.3
Thompson (26), 2000	1995–1999	England	STD clinic	71.0
Kortekangas-Savolainen and Vourinen (27), 2007	1994–1996	Finland	STD clinic	18.7
	2000–2002			25.9
Janier et al (28), 2006	1999–2002	France	STD clinic	14.5
Filén et al (29), 2004	2001–2002	Sweden	STD clinic	72.0
Ramaswamy et al (30), 2004	2002	England	STD clinic	9.0
Buxbaum et al (31), 2003	2003	Germany	STD clinic	22.0
Aryee et al (32), 2005	2004	Gambia	STD clinic	15.0
Hope-Rapp et al (33), 2010	1995–2005	France	STD clinic	37.0
Pena et al (34), 2010	2007	United States	Private laboratory	32.0

STD Sexually transmitted disease

transmission (9). Change in sexual practices are often cited, but few studies have examined the sexual repertoire of adults according to age groups. Although oral sex is reported to be frequent in adolescents and young adults, we found only one study reporting the frequency of these practices in older adults (10). This study confirmed that persons practicing fellation and cunnilingus are significantly younger than those who do not practice them.

The present study had several limitations. The samples were submitted with limited clinical data and it was not known if the samples were collected from a primary episode or a recurrence. The detection method used (viral culture), although very specific, is not the most sensitive. The use of a molecular method would have increased the recovery of HSV (11). Whether the use of a molecular method would have altered the ratio of HSV1/HSV2 is unknown.

We compared our data with those presented in Forward and Lee's study (8), after adjusting our age groups. Overall, the rate of occurrence of HSV1 was lower in male patients from Nova Scotia (New Brunswick 55% versus Nova Scotia 36.7%, P<0.002). The rates for female patients were also different (New Brunswick 63.8% versus Nova Scotia 58.1%, P=0.034). As in the study by Forward and Lee, female patients were over-represented, constituting approximately 80% of all patients tested for all age groups in both studies.

Although some differences exist, our study confirms Forward and Lee's findings that in Atlantic Canada, HSV1 is the main cause of genital herpes. Based on these two studies, however, HSV1 occurrence rates are lower in Nova Scotia than in New Brunswick.

The study by Forward and Lee (8) and the present study differed in several ways. Forward and Lee's study used a single cell line (A549) kept for seven days in a stationary cell tube. We used four cell lines kept for 10 days, including the Mink lung cell line. The Mink lung cell line is reported to be more sensitive than Vero and MRC-5 for the recovery of HSV in culture (12). We used a spin-amplified microplate cell culture assay that was also reported to increase the recovery of viruses (13,14). Whether differences in culture methods preferentially affected the recovery of HSV1 compared with HSV2 is unknown.

The two studies are separated by eight years, and a change in sexual practices over time cannot be excluded. Fellatio and cunnilingus are reported to be more common in younger age groups (15), being perceived as safer than intercourse and as a means of averting pregnancy (16). Such a change would favour the transmission of HSV1 in this population. Forward and Lee's study and the present study indirectly support this observation. Furthermore, most adolescents have no HSV1 antibodies (17), making them susceptible to a primary genital infection with this organism. Whether the susceptibility to HSV1 primary genital infection has varied over time (eg, 1998 to 2001 versus 2006 to 2008) or location (Nova Scotia versus New Brunswick) is unknown. Access to medical care may have differed between Nova Scotia and New Brunswick, and also during the two study periods.

No other published studies based on direct detection concerning the role of HSV1 in genital herpes in Canada were found, although regional differences were mentioned (18). The relative importance of HSV1 in genital herpes varies considerably in other areas of the world (Table 2). Most published studies include a greater proportion of female patients, similar to the present study. Although not meant to be exhaustive, the review of the published studies in the present report found HSV2 to be the main cause of genital herpes.

CONCLUSION

In New Brunswick, HSV1 is the dominant type of HSV detected in samples collected from genital sites. Significant rate differences are seen between the 10 to 39 years of age and the ≥40 years of age groups. Our data confirm the findings of a similar study conducted in Nova Scotia (8). We encourage other Canadian laboratories to reassess the role of HSV1 in the etiology of HSV genital lesions.

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