Genital Herpes Simplex Virus Type 1 in Women: Detection in Cervicovaginal Specimens from Gynecological Practices in the United States

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Received 8 July 2009/Returned for modification 9 October 2009/Accepted 9 November 2009

Herpes simplex virus types 1 and 2 (HSV-1 and -2) are significant human pathogens causing clinically indistinguishable facial and genital lesions. Recently, the number of reported genital herpes cases caused by type 1 virus has increased. Identifying the HSV type is of clinical importance to determine proper treatment, as there is no licensed vaccine or cure. We assessed, by PCR, the frequency of HSV-1 and HSV-2 present in more than 60,000 clinical cervicovaginal specimens derived from samples originating from 43 continental U.S. states. Fourteen percent were positive for HSV-1 and/or HSV-2. This likely represents subclinical shedding. It was not a measurement of the prevalence of HSV infection. While the majority were HSV-2, 32% were HSV-1. The distribution of HSV types varied between the states with the largest number of specimens, New Jersey, Florida, and Texas. Specimens from women under the age of 24 had an HSV-1 positivity rate of 47 percent. Importantly, in New Jersey, an observed age effect was the disproportionately high prevalence of genital HSV-1 in young women. This represents the largest analysis of HSV types reported and has important public health implications, particularly for younger women.

MATERIALS AND METHODS

Clinical specimens. The clinical specimens included in this study were submitted to our (MDL) clinical diagnostic laboratory for HSV testing from January 2007 to December 2007 mainly by private obstetrics and gynecology practices. Most samples were derived from women during general wellness visits (such as annual checkups) and patient self-selection visits. We are unable to ascertain whether the prevalence of HSV in this population approaches that of the general U.S. population. In addition, it is unknown whether these women were suspected of having HSV. Thus, the percentage of random samples versus the number of tests ordered by physicians who knew of or suspected genital HSV is unknown. It is also not known how many of the HSV-positive samples represent primary infections, reactivating clinical recurrences, or asymptomatic shedding. Type-specific tests were performed on each sample following the guidelines of the laboratory’s federal, state, and Clinical Laboratory Improvement Amendments (CLIA) certifications. Patient anonymity was strictly protected in accordance with the federal Health Insurance Portability and Accountability Act (HIPPA) of 1996. None of the samples were accompanied by information regarding the medical history or clinical presentation at the time of specimen collection. Only the patient’s age and state of residence were obtained for the purpose of this study.

Specimen sampling. Cervicovaginal specimen sampling was performed using Oneswab swabbing containers (MDL, Hamilton, NJ), which include a Dacron-based, flocked sampling device plus transport medium optimized for extraction.
TABLE 1. MDL statistics on genital herpes simplex virus in women in 2007

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. (%) of clinical samples</th>
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<tbody>
<tr>
<td>Total specimens...........................................61,376</td>
<td></td>
</tr>
<tr>
<td>Specimens with no gender listed................................329</td>
<td></td>
</tr>
<tr>
<td>Specimens from men...........................................1,012</td>
<td></td>
</tr>
<tr>
<td>Total specimens from women...................................60,035 (98)</td>
<td></td>
</tr>
<tr>
<td>HSV-positive women*............................................8,294 (14)</td>
<td></td>
</tr>
<tr>
<td>Positivity rates in women b</td>
<td></td>
</tr>
<tr>
<td>HSV-1 positive...........................................2,633 (32)</td>
<td></td>
</tr>
<tr>
<td>HSV-2 positive...............................................5,649 (88)</td>
<td></td>
</tr>
<tr>
<td>HSV-1 and HSV-2 positive....................................12 (0.15)</td>
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* Samples were positive for HSV-1 and/or HSV-2.

b Positivity values were determined by real-time PCR analyses. Samples were positive by real-time PCR using separate probes specific for HSV-1 and HSV-2.

of nucleic acids (1). Infectious HSV-1 and HSV-2 have recently been isolated from HSV-positive OneSwab specimens, validating this platform (12). Clinicians were instructed in proper specimen collection technique, which is very important for identifying pathogens using extracted DNA. The initial step involved preparation of the area. Excess secretions or discharge were removed by wiping with a standard sterile swab, which was then discarded. Next, a OneSwab tip was used to firmly, yet gently, sample the mucosal membrane of the vaginal vault by rotating 360 degrees for 10 to 30 s. This ensured adequate cervicovaginal sampling. Due to confidentiality, we cannot control for repeat or noncervicovaginal swabbings being submitted. In the final phase of the sampling, the OneSwab was removed after swabbing and placed into its transport vial. At this time, the shaft was snapped off to completely fit the OneSwab into the vial. It is necessary that the OneSwab fits completely into the vial prior to capping in order to prevent leakage of the specimen. Specimens were transported at room temperature for analysis within 24 h of sampling. Information describing the clinical presentation or any patient identifiers were not provided. These anonymous samples represent discarded material and are only referred to by arbitrary numbers.

Nucleic acid analyses. DNA extraction from the samples, real-time PCR amplification, and determination of cycle threshold (Ct) values using probes specific for glycoprotein B of HSV-1 and HSV-2 were performed exactly as previously described (1). Amplification was performed using Rotor-Gene 3000 instruments (Corbett Research, Mortlake, Australia). PCR results were matched to four criteria: virus type and patient’s sex, age, and state of residence. The values for virus type were further subdivided into cases in which specimens were positive for both HSV-1 and HSV-2. Only specimens identified as being from women were considered in the study; neither unknown-gender nor male samples were analyzed. Statistical analyses were performed using Student’s t and chi-squared algorithms, as appropriate.

RESULTS

Percentage of genital HSV positivity values in our study. We assessed, by real-time PCR analysis, the frequency of HSV-1 and HSV-2 present in 61,376 clinical cervicovaginal specimens submitted to our laboratory (Table 1). The number of specimens from women represented 98% of this total. Fourteen percent of these were positive for either HSV-1 alone, HSV-2 alone, or both HSV-1 and HSV-2 based on real-time PCR. In a previous small-cohort analysis (4,581 total specimens) by us, we observed 11% HSV positivity rates for HSV-1 and/or HSV-2 (1). At first glance, our 14% positivity value from this new large analysis seems comparable to the results of a focused (Puget Sound area) study (10,974 total specimens) which observed a 16% positivity rate (16). However, this Seattle cohort was specifically derived from specimens from individuals who were known to have genital herpes; some even had clinical lesions. As described in Materials and Methods, our sample population was not focused exclusively on patients previously known to have genital HSV disease.

Detection of genital HSV-1 in U.S. women. Our analysis involves 8,294 HSV-positive specimens from women. To our knowledge, this represents the largest HSV-positive cohort study to date. Within this group of women, 68% were positive for HSV-2 and 32% were positive for HSV-1. This amount of the HSV-1 positivity value is slightly greater than that found in our previous small cohort reported in 2005, which had a 28% HSV-1 positivity rate. While the total numbers of HSV-positive specimens differed by almost 20-fold between these two studies and this slight increase might reflect ordering patterns, these new results may suggest a trend of increasing HSV-1 positivity values over the last 4 to 5 years. It was also observed in this new study that 0.15% of women were positive for both HSV-1 and HSV-2. This value represents only 12 positive out of a cohort of over 8,000 women and emphasizes the need to investigate large numbers of specimens. Small-cohort or geographically focused studies likely are not sufficient to capture data on HSV-1 and HSV-2 coinfections.

Percentages of genital HSV-1 positivity rates vary with geography. The group analyzed above, of which 14% were positive for HSV-1 and/or HSV-2 from 60,035 specimens, was derived from samples originating from 43 continental U.S. states. The next goal was to assess the distribution of genital HSV-1 in women based on geographical location. We focused on the three states with the largest number of specimens, New Jersey, Florida, and Texas. The total HSV-positive specimens from these states represent 50% of the total positive samples (Table 2). In this subgroup, the number of HSV-1-positive samples was 31%, consistent with the entire cohort (Table 1). Both Florida and Texas had HSV-1 positivity values, 30% and 26%, respectively, which were below our national average of 32% described above (P < 0.05). New Jersey, on the other hand, had values which were approximately 10% greater than the national average (P < 0.05). In this group, Texas had the largest number of samples and the lowest number of HSV-1-positive cases. These observations indicate that there is not a sampling bias toward HSV-1 positivity rates due to the large number of specimens tested. Based on these observations, we conclude that there exist geographical variations in the extent of genital HSV-1 positivity values in women in the United States. Based on the three states with the largest number of samples in our study, this variation extends from 26 to 41%.

Percentages of genital HSV-1 positivity vary with age. In the next portion of this analysis, we focused on samples originating in all states. The distribution of HSV positivity values was broken down by age, roughly by generation. Specimens representing 93% of the total HSV-positive women were studied (Table 2). Samples which were positive for both HSV-1 and HSV-2 were detected in all age groups. While the entire group had an HSV-1 positivity rate of 31%, there was a striking distribution with age. Importantly, specimens from women under the age of 24 had an HSV-1 positivity value of 47%. As the groups increased with age, the HSV-1 positivity rates decreased from 47% to 29% to 22% and 19%. Thus, the most statistically significant difference in the genital HSV-1 positivity rates was between those women who were ≤24 years of age and those >24 years of age. From this analysis, it appears that
there may be three separate groups. Women aged 45 and older had an HSV-1 positivity rate of 19 to 22%, while those between 25 and 44 were intermediary at 29%. Based on these findings, we conclude that a major contributor to the number of cases of genital HSV-1 is the high incidence in women under 24 years of age.

The highest percentages of genital HSV-1 positivity are in younger women. Relevant to this finding was our observation that of the HSV-positive samples from women in New Jersey, 41% were positive for genital HSV-1 (HSV-1 positivity) (Table 2). To validate our conclusion described above, we analyzed the results for HSV positivity rates in New Jersey by age (Table 2). As predicted by our analysis of the nationwide samples (Table 2), women aged 24 years and younger in New Jersey had an HSV-1 positivity value of 64%. Based on this finding, we conclude that the observed age effect is due to the disproportionately high prevalence of genital HSV-1 in young women in New Jersey. Women in New Jersey aged between 25 and 64 had HSV-1 positivity rates of 32 to 35%, consistent with the national average (Table 1). Similarly, New Jersey women aged 65 and older were 21% HSV-1 positive, which is also consistent with the national average for that age group (Table 2). These findings support the hypothesis that the high level of HSV-1 positivity rates in women under 24 years of age, compared to the national average for all ages, is the driving force behind apparent increases in genital HSV-1 positivity values. The most important lesson from this analysis is that there exists a statistically significant difference between the genital HSV-1 positivity rates in women ≤24 years of age and those >24 years of age.

DISCUSSION

To our knowledge, this analysis represents the largest survey of HSV types reported to date. In a previous small-cohort analysis (4,581 total specimens) by us, we observed 11% HSV positivity rates for HSV-1 and/or HSV-2 (1). It should be noted that we have recently validated coinfections of HSV-1 and HSV-2 in clinical specimens from OneSwab devices using virus growth, plaque purification, and rapid immunotyping methodologies in cultured cells (2, 12). These data have important public health implications, particularly for younger women, since the transmission of genital HSV-1 from mother to neonate may be highly efficient (3). It should be emphasized that the clinical specimens that were analyzed were derived from women during general wellness visits (such as annual check-ups) and patient self-selection visits. It is recognized that these samples are neither completely random nor representative of the general U.S. female population. It is not known how many of these specimens from HSV-positive women were random samples or how many of their physicians knew of or suspected genital HSV. With regard to additional HSV epidemiology issues, it is also unknown how many of the HSV-positive samples represent primary infections, reactivating clinical recurrences, or asymptomatic shedding. These results do, however, reflect the status of a large number of women who received...
Considering the nonselected nature of our population with respect to their prior genital HSV status, our genital HSV-1 positivity rate of 14% is much higher than might be expected based on previous literature (6, 8, 9, 11, 14–16). Previous analyses of the prevalence of HSV-1 may be limited by their focus on individuals with genital HSV disease. In addition, we now present evidence that age is an important determinant in genital HSV-1 positivity values. The significance of our data is that it implies that there may be a new silent epidemic under way, which is likely being driven by reduced sociological sensitivities regarding what constitutes sexual activity, particularly in the younger population.

Our specimen collection was such that half of the samples originated within three states, while the others were generally spread out among 40 other states. Thus, it is not a random national group. Focusing on the samples from New Jersey, Florida, and Texas, we observed differences in the percentages of HSV-1 positivity values within each state subcohort. In particular, it was observed that women in New Jersey had an HSV-1 positivity rate that was almost 10% higher than the national average. We conclude that the observed age effect is due to the disproportionately high prevalence of genital HSV-1 in young women in New Jersey. Our number of 998 HSV-positive samples from New Jersey is much higher than most other previous studies have reported (14–16). Nevertheless, our New Jersey cohort is consistent with all of these other “localized” cohorts, which had HSV-1 positivity rates approaching 50%. Thus, geography appears to impact the incidence of genital HSV-1.

The most significant conclusion from this study is that women under 24 years of age have higher HSV-1 positivity rates than older women. It is important to note that we are not measuring the prevalence of HSV infection in this population of women. Our data likely represent a measurement of subclinical shedding. Individuals with genital HSV-2 infections display higher HSV-1 infection frequency. The increased genital HSV-1 positivity values. The significance of our data is that it implies that there may be a new silent epidemic under way, which is likely being driven by reduced sociological sensitivities regarding what constitutes sexual activity, particularly in the younger population.

This is the first reported indication that genital HSV-1 positivity rates in the United States are highest in young women. Consideration of this information would benefit physicians providing primary gynecological and obstetric care to this population of women. Because genital HSV-1 infections have a milder clinical presentation, lower risk of recurrence, and higher risk for transmission from mother to infant during delivery than HSV-2, this knowledge is important for physicians counseling patients with genital herpes and guiding the management of HSV infections. It will be important for us to track this trend in genital HSV-1 longitudinally to ascertain whether it is maintained.

ACKNOWLEDGMENTS

We thank Janet Cohen of MDL for assistance in compiling data and Tevfik Dorak of Humigen LLC, Hamilton, NJ, for statistical assistance and helpful comments. These studies were supported by MDL.

REFERENCES