Detection of varicella zoster virus in genital specimens using a multiplex polymerase chain reaction

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Objective: To compare the relative proportions of varicella zoster virus (VZV) and herpes simplex viruses in specimens obtained from the genital lesions of adults presenting with presumed genital herpes infection.

Methods: Swabs of genital lesions from 6210 patients attending general practices, infectious diseases clinics within hospitals, or sexual health centres for treatment of their genital lesions were tested using polymerase chain reaction (PCR) technology. The multiplexed PCR was capable of detecting herpes simplex virus types 1 and 2 (HSV-1, HSV-2), VZV, and cytomegalovirus in a single sample.

Results: A total of 2225 patients had viruses detected by PCR. HSV-1 was detected in 36%, HSV-2 in 61%, and VZV in 2.9% of PCR positive samples. Of the 63 patients with VZV genital infection, many were thought to have HSV infection before laboratory testing.

Conclusions: The finding of VZV in nearly 3% of virus positive genital specimens demonstrates that this virus needs to be considered as a differential diagnosis for genital herpetic lesions. Advice provided to patients with VZV genital infection regarding the source of infection, likelihood of recurrence, and potential for transmission of the virus will be different from that given to patients with HSV infection.

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aricella zoster virus (VZV) causes chickenpox and, in its recurrent form, shingles. Zoster can occur at all ages, but is more common in those aged over 50 (www.harrisonsonline.com). Individual cases of recognised vulval and anogenital infection with VZV have been described in adults, and cases of genital infection with this agent in children have also been reported. In some circumstances infections in children have required laboratory confirmation to eliminate accusations of child abuse when infection with herpes simplex virus (HSV) was suspected. However, we are unable to find any reports describing the incidence of VZV in genital lesions of adults presenting with presumed HSV infection.

Polymerase chain reaction (PCR) technology has increased the sensitivity of detection of viruses such as VZV over methods such as virus isolation or direct immunofluorescence. In particular, the slower replication in vitro of VZV compared to HSV by up to 10 days in some cases may result in virus isolation attempts being abandoned as negative when the clinical diagnosis is HSV infection, resulting in the occasional failure to isolate VZV. Historically, therefore, the incidence of VZV associated genital infection is likely to have been underestimated. We describe here the results of a study investigating the incidence of herpesvirus infections in genital specimens using a multiplex PCR that detects and differentiates between HSV-1, HSV-2, cytomegalovirus (CMV), and VZV.

MATERIALS AND METHODS

Patients and specimens
Specimens were received from 6210 patients who attended general practices, infectious diseases clinics within hospitals, or sexual health centres for treatment of genital lesions. With the permission of the patients, swabs of lesions thought to be of viral aetiology were sent to the laboratory for PCR testing. The clinical diagnosis was inferred from the herpesvirus type written on the request form by the clinician ordering the test: VZV, HSV, and either VZV or HSV. If the clinician did not specify any herpesvirus, the clinical diagnosis was recorded as “no diagnosis.” The study was carried out on specimens tested between October 1999 and December 2001.

MULTIPLEX PCR assay to detect herpesviruses
The PCR assay used to detect HSV-1, HSV-2, CMV, and VZV DNA in clinical material has been described previously. Briefly, specimens were vortexed and DNA extracted by treating 40 µl of specimen with 2 µl 1% Nonidet P40 at 70°C for 45 seconds. Nested PCR was performed using primers specific for HSV (both serotypes 1 and 2), HSV-2, CMV, and VZV. Primers specific for equine herpesvirus type 4 were also included to amplify this virus, which was added to each sample at the time of DNA extraction to serve as an internal amplification control. Amplified products were electrophoresed in 2% agarose gels, stained in ethidium bromide and photographed using a Gel Doc 2000 (BioRad, USA). All bands were interpreted qualitatively. A typical gel is shown in figure 1.

Statistical analysis
The proportion of requests where the possibility of VZV was noted was compared to requests where VZV was not indicated using two sample comparisons of proportion.

RESULTS

Proportions of HSV-1, HSV-2, VZV, and CMV in genital specimens
A total of 6210 genital swabs were sent to the laboratory for testing using the multiplex herpesvirus PCR during the period October 1999 to December 2001 (table 1). At least one of the viruses represented in the assay was found in 2255 of these specimens (36.3%). HSV-2 was the most common virus detected (61% of all positives); HSV-1 was present in 36% of specimens and VZV in 2.9% (table 1). CMV was seen in low numbers (five of 2255 positive samples (0.2%)). All of the CMV positive specimens were from females (result not shown). In these cases the CMV DNA detected was assumed to represent urine contamination of the specimen rather than to implicate CMV as a causative agent of genital infection.

Patients positive for VZV DNA
The age range, sex, and diagnosis at presentation of the 65 patients (25 male, 40 female) with genital lesions positive for VZV DNA is shown in table 2. Of the 25 males positive for VZV
of 65 patients whose genital specimens contained VZV DNA

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Patients (males, females)</th>
<th>VZV</th>
<th>VZV and HSV</th>
<th>HSV</th>
<th>None†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>7 (2, 5)</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>16–50</td>
<td>37 (17, 20)</td>
<td>5</td>
<td>13</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>21 (6, 15)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>65 (25, 40)</td>
<td>5</td>
<td>10</td>
<td>16</td>
<td>34</td>
</tr>
</tbody>
</table>

*Patients with genital lesions and VZV and/or HSV included in the differential diagnosis.
†Patients with genital lesions but no diagnosis recorded.

DISCUSSION

Of 2255 specimens positive for at least one of HSV-1, HSV-2, VZV, and CMV, 65 (2.9%) were positive for the presence of VZV DNA. Although some of these infections were diagnosed at the time of presentation as being likely to be caused by VZV, more were thought to be cases of HSV infection. Our study highlights that a small but significant number of presumed genital HSV infection are caused by VZV, and that
The detection of VZV in genital specimens highlights the sensitivity and versatility of molecular based techniques such as PCR, which are being increasingly used in virus diagnostic laboratories. These tests are replacing virus isolation methods which, although sensitive for the detection of HSV-1 and HSV-2 within an a short time frame (4–7 days), are less so for VZV which takes longer to replicate. While the incidence of VZV associated genital infection was low in our population compared to HSV-1 and HSV-2 infection, it was not the expected causative agent in the majority of the 65 patients in whom it was identified. Advice provided to patients regarding the source of their infection, likelihood of recurrence, and potential for transmission of VZV, and their response to this advice, is likely to be substantially different from that given to patients with HSV-1 or HSV-2 infection. Thus, doctors and virologists should consider the possibility of VZV in patients presenting with genital infection, and test accordingly.

**CONTRIBUTORS**
CJB, senior scientist with responsibility for diagnostic testing in the area described, input into the design, validation, and performance of the PCR, responsible for preparation of the manuscript; JDD, senior scientist with input into the design, validation, and performance of the PCR, and assistance with collation of data for input into the manuscript; LMACG, statistician, and assistance with preparation and review of the manuscript; TR, clinician, with special expertise in the diagnosis of sexually transmitted infections, input into preparation and review of the manuscript.

**REFERENCES**