Diagnosis of herpes simplex virus-1 keratitis using Giemsa stain, immunofluorescence assay, and polymerase chain reaction assay on corneal scrapings

S Farhatullah, S Kaza, S Athmanathan, P Garg, S B Reddy, S Sharma

Aims: To evaluate three tests used routinely for the diagnosis of herpes simplex virus (HSV) keratitis.

Methods: Corneal scrapings from 28 patients with clinically typical dendritic corneal ulcer suggestive of HSV keratitis, and 30 patients with clinically non-viral corneal ulcers, were tested by (i) Giemsa stain for multinucleated giant cells, (ii) immunofluorescence assay (IFA) for HSV-1 antigen, and (iii) polymerase chain reaction (PCR) for HSV-1 DNA, by investigators masked to clinical diagnosis. The control subjects were also investigated by smears and cultures for bacteria, fungus, and Acanthamoeba.

Results: The specificity and positive predictive values of all three tests for the diagnosis of HSV keratitis were between 95–100%. The sensitivity of IFA and PCR was 78.6% and 81.2%, respectively, and the difference was not significant; however, their sensitivity and negative predictive value were significantly higher than Giemsa stain.

Conclusions: While a combination of IFA and PCR constitute the choice of tests in clinically suspected cases of HSV keratitis, multinucleated giant cells in Giemsa stain can preempt testing by IFA and PCR in otherwise atypical cases of HSV keratitis.

RESULTS

There were 20 men and eight women in the test group and 16 men and 14 women in the control group. The mean age was

Herpes simplex virus (HSV) keratitis is a diagnostic and therapeutic challenge to the ophthalmologist. Virus isolation is considered a standard procedure for diagnosis of viral infections; however, it is a relatively time consuming procedure and depends on viable infectious material that usually needs to be transferred to a special virology laboratory for processing. Rapid diagnostic tests, that allow results within hours, have been reported in the past two decades. These rapid tests have been either enzyme or fluorescence based immunological detection of HSV-1 antigen or polymerase chain reaction (PCR) based detection of viral DNA. None of these studies evaluated these tests simultaneously in a clinically well defined group of patients.

For the past 5 years we have been routinely employing a trial of tests on corneal scrapings for the diagnosis of HSV-1 keratitis in patients seen at our institute, which is a tertiary eye care centre located in south central India. The three tests include Giemsa stain, immunofluorescence assay (IFA), and PCR. The purpose of this study was to evaluate the efficacy of these three tests in the diagnosis of HSV-1 keratitis.
The authors achieved an overall confirmed laboratory diagnosis in only 40% of the clinically suspected patients. This indicates the difficulty in establishing laboratory diagnosis in cases suspected to be viral keratitis and the need for more sensitive techniques. PCR based techniques have been employed on tear samples, corneal epithelium, and corneal buttons for the investigation of herpetic ocular diseases with a promise of greater sensitivity and specificity compared with antigen detection and viral culture methods. Although mentioned in textbooks, the older but simpler method of detection of multinucleated giant cells, lymphocytes, and inclusion bodies in Giemsa stained corneal scrapings, has not found much favour in the diagnosis of HSV keratitis. However, we recently reported its use in the diagnosis of HSV keratitis in a contact lens wearer.

Keen to find an optimum combination of laboratory tests that may help to establish the diagnosis of HSV keratitis in suspected or atypical cases of keratitis, this study was undertaken. We chose the gold standard of clinically typical cases of HSV epithelial keratitis for evaluation of these three tests as viral isolation techniques have not been very successful in our hands. Our laboratory has been engaged in using these three tests on corneal scrapings for the diagnosis of HSV keratitis and based on our clinical correlation we consider positivity in two of the three tests essential for a confirmed diagnosis. Although a clinical diagnosis of HSV-1 epithelial keratitis is relatively straightforward, the clinical features in stromal HSV keratitis can often be obscure, which may further be confounded by superimposed infection with bacteria or fungus in countries such as India. The results of this study indicate all three tests to be highly specific with very high positive predictive values.

We found PCR to be most sensitive, although the difference in sensitivity from IFA was statistically not significant. There was only one sample in the control group that was considered positive by IFA but there were six cases that were negative in the test group (table 1). In our hands IFA showed better sensitivity than an earlier reported study; however, we are wary of diagnosis based on a single test and use PCR to confirm or strengthen the diagnosis. False positivity is known to be a common problem with PCR based tests and during routine use, one is likely to face the problem of amplicon contamination. However, the presence of DNA in negative controls invalidates a test run and requires retesting. This study confirms the excellent specificity of the assay. Its sensitivity is higher than any other currently employed test; nevertheless, sample variation may account for absence of DNA in two cases among our test group of patients accounting for less than 100% sensitivity.

Although the Giemsa stain did not pick up any case that was missed by IFA or PCR, we consider it a valuable test. It can provide a clue to the presence of HSV infection in an otherwise unsuspected case. Inclusion of Giemsa stain of corneal scraping is a routine procedure in the investigation of all patients seen in our cornea clinic with suspected microbial keratitis, and we recommend testing of additional corneal scrapings by IFA and PCR in all cases where MNGCs, inclusion bodies, or lymphocytes are seen in the Giemsa stained scraping. This procedure may help clinch the diagnosis in cases with atypical presentations. IFA and PCR remain the tests of choice in all suspected cases of HSV keratitis.

**DISCUSSION**

A prevalence of 7.8% has been reported earlier for HSV keratitis among patients attending cornea clinic in a tertiary eye care centre in south India. The study included 234 out of 300 patients having clinical features suggestive of viral aetiology. Despite using procedures of viral culture and IFA, the authors achieved an overall confirmed laboratory diagnosis in only 40% of the clinically suspected patients. This indicates the difficulty in establishing laboratory diagnosis in cases suspected to be viral keratitis and the need for more sensitive techniques. PCR based techniques have been employed on tear samples, corneal epithelium, and corneal buttons for the investigation of herpetic ocular diseases with a promise of greater sensitivity and specificity compared with antigen detection and viral culture methods. Although mentioned in textbooks, the older but simpler method of detection of multinucleated giant cells, lymphocytes, and inclusion bodies in Giemsa stained corneal scrapings, has not found much favour in the diagnosis of HSV keratitis. However, we recently reported its use in the diagnosis of HSV keratitis in a contact lens wearer.

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**Table 1** Results of Giemsa stain, IFA, and PCR in test and control subjects

<table>
<thead>
<tr>
<th>Test method</th>
<th>Test group (n = 28)</th>
<th>Control group (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Giemsa stain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNGC+</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>MNGC−</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>Not done</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>IFA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1 antigen +</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>HSV-1 antigen −</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td><strong>PCR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1 DNA +</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>HSV-1 DNA −</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

+ = Detected, − = not detected.
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