Epidemiology and molecular analysis of herpes simplex keratitis requiring primary penetrating keratoplasty

B C Branco, P A Gaudio, T P Margolis

Aims: To determine whether herpes simplex keratitis (HSK) has declined as an indication for penetrating keratoplasty (PKP) at the University of California San Francisco (UCSF) over the past 30 years.

Methods: Records of the Hogan Eye Pathology Laboratory were reviewed to determine the incidence of PKP performed for HSK from 1972 through 2001. Archived corneal tissue with the diagnosis of HSK was evaluated for herpes simplex virus (HSV) DNA by polymerase chain reaction (PCR) based assays.

Results: The number of corneal buttons submitted with the clinical diagnosis of HSK decreased from 1972 to 2001, while the overall number of PKPs performed did not. The percentage of corneal buttons with a clinical diagnosis of HSK that contained detectable HSV DNA did not change over the course of the study period.

Conclusion: HSK declined as an indication for PKP from 1972 to 2001 at UCSF. It is unlikely that this decline was the result of improved diagnostic accuracy since detection of HSV DNA in corneal buttons with a clinical diagnosis of HSK was similar at a beginning and end of the study period.

Corneal scarring as consequence of viral keratitis has declined as an indication for penetrating keratoplasty (PKP) over the past five decades. During the 1950s, viral keratitis was among the most common indications for corneal transplant, accounting for 19.7% and 25.7% of PKPs performed at major referral centres in Los Angeles and Baltimore, respectively. During the following three decades, the annual number of PKPs being performed at major referral centres for viral keratitis remained largely unchanged, but with increased numbers of PKPs being performed for other conditions, such as pseudophakic bullous keratopathy and keratoconus, viral keratitis dropped as a leading indication for PKP. This changed, however, in the 1990s, when referral centres reported a marked decline in the number of PKPs being performed for viral keratitis. Improvements in the medical management of herpes simplex virus (HSV) and varicella zoster virus (VZV) keratitis are probably responsible for the recent decline in the number of PKPs performed for these conditions. A second possible explanation is that improvements in diagnostic accuracy have led to fewer eyes assigned the diagnosis of viral keratitis. For example, acanthamoeba keratitis, which is easily mistaken for HSV keratitis (HSK), was not well recognised until the mid 1980s. It is also possible that the recent decline in the number of PKPs being performed for viral keratitis is consumer driven, with fewer patients electing to have corneal grafts as surgical outcome data for eyes with viral keratitis have become available.

It has been our impression that over the past decade PKP is only rarely performed for patients with HSK at the University of California, San Francisco (UCSF). The current study was carried out in order to determine whether there has been a decrease in the incidence of PKPs performed for HSK over the past three decades at UCSF. Since there is no established gold standard for making a clinical or pathological diagnosis of HSK, molecular testing of archived corneal buttons for HSV was performed as an independent diagnostic measure. To our knowledge this is the first study to evaluate changing trends in the indications for PKP where the diagnosis of HSK was evaluated independent of other viral causes of corneal scarring.

MATERIALS AND METHODS

Review of pathological records

We reviewed the records of all corneal buttons submitted from 1972 to 2001 to the Hogan Eye Pathology Laboratory at the UCSF. The resultant data were used to determine the total number of PKPs performed during the study period, as well as the number of primary PKPs performed for a clinical diagnosis of HSK. We chose to only include primary, and not subsequent, grafts for HSK in our analysis since the indication for this small number of re-grafts (four) was not always clear from the clinical record (for example, role of recurrent HSV v graft rejection).

For all archived specimens included in this analysis, we collected the following data: age, sex, date of surgery, the presence or absence of multinucleated cells, blood vessels, stromal loss, inflammatory cells, disruption of the Descemet's membrane, and endothelial loss. Pathology records were also reviewed in order to identify corneal buttons as negative controls for molecular diagnostic testing. This group of controls included corneal tissue from primary PKPs performed for keratoconus, bacterial keratitis and fungal keratitis. This study was approved by the UCSF committee on human research.

Molecular diagnostics

Pieces of 15–20 mg of each archived, formalin fixed, tissue block were deparaffinised with xylene and washed with ethanol in preparation for DNA extraction. DNA was extracted using the DNeasy Tissue Kit for Animal Tissues (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) amplification for HSV DNA (types 1 and 2) was performed as previously described. In brief, amplification reactions were performed in a 100 μl reaction volume consisting of the following: 197 nM of each primer (table 1); 5 μl of extracted corneal DNA, 1X PCR buffer (Sigma, St Louis, MO, USA), 50 μM each dNTP (PE Biosystems, Foster City, CA, USA), 2.5 mM magnesium...
chloride and 2.5 U RedTaq DNA polymerase (Sigma). Thermal cycling was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) using the following programme: initial denaturation for 2 minutes at 94°C followed by 41 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension for 2 minutes at 72°C.

PCR amplification for acanthamoeba DNA was performed as previously described in a 50 µl reaction volume consisting of the following: 500 nM of each primer (table 1), 10 µl of extracted corneal DNA, 1X PCR Buffer (Sigma), 100 µM of each dNTP (PE Biosystems), 4 mM of magnesium chloride, and 2.5 U of RedTaq DNA polymerase (Sigma). Thermal cycling was performed using the following programme: initial denaturation for 2 minutes at 94°C, followed by 46 cycles of 94°C for 60 seconds, 54°C for 30 seconds, and 60°C for 60 seconds, followed by a final extension for 2 minutes at 72°C. Amplification products were resolved on a 4% polyacrylamide gel and visualised by ethidium bromide staining.

Purified viral HSV-1 DNA (ABI, Columbia, MD, USA) and a cloned 229 bp acanthamoeba DNA target sequence served as positive controls for PCR. Assay sensitivity was determined using control target DNA mixed in with archived tissue from corneas with keratoconus.

In order to minimise sample contamination, a new blade was used for each tissue sample. In order to control for reagent contamination, control corneas were processed in parallel with experimental tissues.

RESULTS

From 1972 through 2001, 4207 corneal buttons were submitted to the Hogan Eye Pathology Laboratory at UCSF. Seventy six of these corneal buttons were from eyes with HSK undergoing primary PKP. The median age of these HSK patients was 49 years (range 6–82 years). The pathological features of the corneal buttons included inflammatory cells (87%), endothelial cell loss (71%), vascularisation (59%), and rupture of Descemet’s membrane (25%) (table 2). Only four corneas (5%) had multinucleated giant cells. Sixty six per cent of the corneas with vascularisation had vessels in the deep corneal stroma.

Epidemiological data on primary PKPs performed for HSK from 1972 through 2001 are summarised in table 3. There was a steady decline over time in both the number and percentage of corneal buttons submitted with HSK as the indication for primary PKP. As analysed by decade, HSK was the indication for PKP in about 7% of corneal buttons submitted from 1972–81, 2% of corneal buttons submitted from 1982–91, and 1% of corneal buttons submitted from 1992–2001. These differences were all statistically significant (χ² p<0.001). Between 1972 and 2001, four of the corneal buttons with a diagnosis of HSK were not from primary PKPs. Data from these four buttons were not included in our analysis.

As an independent diagnostic measure of HSK, archived corneal tissue with a clinical-pathological diagnosis of HSK was assayed for HSV DNA by PCR. Since acanthamoeba keratitis is recognised as a mimic of HSK, archived tissue was also assayed for acanthamoeba DNA. Assay sensitivity for both sets of target DNA was between 10 and 100 gene copies per 20 mg of corneal tissue. Archived tissue from 34 of 76 corneas with a diagnosis of HSK was available for analysis. HSV DNA was detected in 17 of the 34 (50%) archived tissue samples. Analysed by decade, nine of 21 corneas (43%) from 1972–81, three of four corneas (75%) from 1982–91, and five of nine corneas (55%) from 1992–2001 were positive for HSV DNA. There was no statistical difference in the percentage of corneas from each decade that were positive for HSV DNA (p>0.05 for all comparisons; two tailed Fisher’s exact test for multiple comparisons), although this may have been a consequence of the relatively small sample sizes. Acanthamoeba DNA was not detected in any of the 34 archived corneas available for PCR analysis. We detected no HSV or acanthamoeba DNA in 11 control corneas; this included four corneas with a diagnosis of bacterial keratitis, two with a diagnosis of fungal keratitis, and five with a diagnosis of keratoconus.

Table 1 Primers used for PCR amplification of herpes simplex virus (HSV) and acanthamoeba DNA

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Final length</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>92 bp</td>
<td>Forward strand: 5’- CATCACCGACCAGCGAGGGAAGGAC</td>
</tr>
<tr>
<td>Acanthamoeba</td>
<td>229 bp</td>
<td>Reverse strand: 5’-GGGGAGAAGCCTGGTTGTTGTA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Years</th>
<th>Number received</th>
<th>Multinucleated giant cells</th>
<th>Inflammatory cells</th>
<th>Vascularisation</th>
<th>Deep vascularisation</th>
<th>Ruptured Descemet’s membrane</th>
<th>Endothelial cell loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>72–6</td>
<td>26</td>
<td>1 (4)</td>
<td>23 (88)</td>
<td>19 (73)</td>
<td>14 (54)</td>
<td>9 (35)</td>
<td>16 (62)</td>
</tr>
<tr>
<td>77–81</td>
<td>20</td>
<td>2 (10)</td>
<td>20 (100)</td>
<td>15 (75)</td>
<td>10 (50)</td>
<td>6 (30)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>82–6</td>
<td>13</td>
<td>1 (8)</td>
<td>11 (84)</td>
<td>6 (46)</td>
<td>3 (23)</td>
<td>3 (23)</td>
<td>12 (92)</td>
</tr>
<tr>
<td>87–91</td>
<td>5</td>
<td>0</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td>2 (40)</td>
<td>0</td>
<td>2 (40)</td>
</tr>
<tr>
<td>92–96</td>
<td>6</td>
<td>0</td>
<td>5 (83)</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>0</td>
<td>4 (67)</td>
</tr>
<tr>
<td>96–01</td>
<td>6</td>
<td>0</td>
<td>4 (66)</td>
<td>1 (17)</td>
<td>0</td>
<td>1 (17)</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>4 (5)</td>
<td>66 (87)</td>
<td>45 (59)</td>
<td>30 (39)</td>
<td>19 (25)</td>
<td>54 (71)</td>
</tr>
</tbody>
</table>

Table 2 pathological findings of corneas with a clinical diagnosis of herpes simplex keratitis (%)
slightly greater percentage of corneas with HSK (54–83%).

Investigators have been able to detect HSV-1 DNA in a

and suboptimal storage conditions decreased our ability to
detect this by PCR.

It is likely that more than 50% of the corneal buttons in our
study contained HSV-1 DNA, but that fixation, embedding, and
suboptimal storage conditions decreased our ability to
detect this by PCR.

One limitation of the current study was that there was only
enough archived tissue to evaluate about 45% of the study
corneas for HSV-1 DNA. This may have introduced sampling
bias since a significantly greater percentage of study corneas
from 1987–2001 were available for analysis than from 1972–
86 (Z test for two proportions; \( p = 0.009 \)). However, we have
yet to come up with a model for how disproportionate
depletion of the older tissue biased our data in favour of the
reported results.

A second limitation of this study was that the clinical
information collected was limited to that supplied by the
surgeon submitting the corneal button. This included (1)
preoperative diagnosis, (2) primary versus repeat PKP, (3)
date of surgery, (4) location of surgery, and (5) surgeon’s
name; the same basic information available in previous
epidemiological studies that have looked at the changing
trends in indications for PKP. Additional clinical information
may have been helpful in confirming the preoperative
diagnosis of HSV keratitis in our study patients, but many
of the clinical charts from the first half of the study (1972–
86) were unavailable for review, the same half of the study
period in which ~78% of the grafts were performed. Thus, in
designing this study we specifically chose to avoid the
significant sampling bias that this issue would introduce by
collecting just those clinical data submitted along with the
corneal button.

The findings of this study suggest that there has been a
recent decline in the number of cases of HSV keratitis that
progress to visually significant scarring. It is tempting to
speculate that this decline is because of recent progress in the
medical management of HSV keratitis. Trifuridine was
introduced into the US marketplace in the mid-1970s.\(^{26-28}\)
This was soon followed by the introduction of aciclovir.\(^{29-33}\) By
the early 1980s a number of published reports had described
the efficacy of these drugs in managing clinical HSV ocular
disease, and their use was becoming more widespread.\(^{34-35}\)
By this time it was also becoming widely appreciated that
HSV should not be treated with potent topical corticosteroids
in the absence of antiviral coverage.\(^{36-38}\) As the use of
prophylactic aciclovir becomes more widely accepted it is likely
that HSV will continue to decline as an indication for PKP.

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us in standardising our PCR assay for acanthamoeba DNA.

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The authors have no financial interest in any products mentioned in this
paper.

**Table 3 Epidemiological and molecular diagnostic data**

<table>
<thead>
<tr>
<th>Years</th>
<th>Total number of corneal buttons</th>
<th>Number and (%) of corneal buttons with diagnosis of HSK</th>
<th>Number of corneal buttons evaluated by PCR</th>
<th>Number and (%) of corneal buttons with HSV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>72–6</td>
<td>367</td>
<td>26 (7%)</td>
<td>8</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>77–81</td>
<td>330</td>
<td>20 (6%)</td>
<td>13</td>
<td>7 (54%)</td>
</tr>
<tr>
<td>82–6</td>
<td>381</td>
<td>13 (3%)</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>87–91</td>
<td>476</td>
<td>5 (1%)</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>92–6</td>
<td>500</td>
<td>6 (1%)</td>
<td>5</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>97–01</td>
<td>420</td>
<td>6 (1%)</td>
<td>4</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Total</td>
<td>2474</td>
<td>76 (3%)</td>
<td>34</td>
<td>17 (50%)</td>
</tr>
</tbody>
</table>

PKP, penetrating keratoplasty; HSK, herpes simplex keratitis; PCR, polymerase chain reaction; HSV, herpes simplex virus.