EXTENDED REPORT

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

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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 the 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.[1 2] 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.[3 4] This changed, however, in the 1990s, when referral centres reported a marked decline in the number of PKPs being performed for viral keratitis.[5–7]

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.[6 8–12] 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.[8]

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 I and II) was performed as previously described.[13–15] 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

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

Pathogen	Final length	Primers
HSV	92 bp	Forward strand: 5'- CATCACCGACCCGGAGAGGGAC Reverse strand: 5'–GGGCCAGGCGCTTGTTGGTGTA
Acanthamoeba	229 bp	Forward strand: 5'- GTTTGAGGCAATAACAGGT Reverse strand: 5'– GAATTCCTCGTTGAAGAT

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[16] 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 (χ^2 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 2 pathological findings of corneas with a clinical diagnosis of herpes simplex keratitis (%)

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

Table 3 Epidemiological and molecular diagnostic data

Years	Total number of corneal buttons	Number and (%) of corneal buttons with diagnosis of HSK	Number of corneal buttons evaluated by PCR	Number and (%) of corneal buttons with HSV DNA
72–6	367	26 (7%)	8	2 (25%)
77–81	330	20 (6%)	13	7 (54%)
82–6	381	13 (3%)	1	0
87–91	476	5 (1%)	3	3 (100%)
92–6	500	6 (1%)	5	3 (60%)
97–01	420	6 (1%)	4	2 (50%)
Total	2474	76 (3%)	34	17 (50%)

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

DISCUSSION

In this study we evaluated the records and corneal pathology specimens of the Hogan Eye Pathology Laboratory from 1972 through 2001. The number of corneal buttons submitted to the laboratory on a yearly basis as well as the diagnosis assigned to these tissues were used to determine the frequency with which HSK was an indication for PKP. The data clearly indicate that HSK declined significantly as an indication for PKP during the study period, most dramatically in the 1980s. One possible explanation for this drop is that clinicians during this time were becoming more aware of other ocular conditions requiring PKP that could mimic HSK (such as acanthamoeba keratitis), and properly assigning these other diagnoses to their surgical cases. To evaluate this possibility we assayed study corneas for both HSV and acanthamoeba DNA. The resultant data do not support the hypothesis that the recent decrease in PKPs performed for HSK was a consequence of improved diagnostic accuracy. HSV DNA was detected in a similar percentage of corneal buttons clinically diagnosed with HSK during the first 10 years of the study period (1972–81) as during the last 10 years (1992–2001). Furthermore, acanthamoeba DNA was found in none of the archived corneal buttons that were available for analysis.

The recent decline in the number of primary PKPs performed for HSK at UCSF is consistent with data reported by other major referral centres of an overall decline in grafts being performed for corneal scarring caused by viral keratitis.[3][6][17] Cosar and colleagues observed a drop of nearly 90% during the 1990s, Lois and colleagues reported a 25% decrease between 1990 and 1995 and Legeais et al noted an 82% drop from the late 1980s to the late 1990s.[5][6][18] An important difference between our work and that of previous investigators is that our study focused exclusively on HSK. This was done so that we could make a definitive statement about recent changing trends in the use of PKP for patients with HSK, a statement that could not be made based on data presented in previous studies in which both HSV and VZV keratitis were grouped into a single category as an indication for PKP. The focused approach of our study is also the likely explanation for why the percentage of PKPs performed for HSK in our study was about half of the number performed for "viral keratitis" in previous studies.

In the current study we found HSV-1 DNA by PCR in 50% of corneal buttons with a clinical diagnosis of HSK. These results are consistent with previous studies of formalin fixed, paraffin embedded tissue in which HSV-1 DNA was detected in 30%–72% of suspect corneal buttons.[19][20] In unfixed tissue, investigators have been able to detect HSV-1 DNA in a slightly greater percentage of corneas with HSK (54–83%).[21–25] 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.[31][34][35] By this time it was also becoming widely appreciated that HSK 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 HSK will continue to decline as an indication for PKP.

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