EXTENDED REPORT

The role of common viral ocular pathogens in Thygeson's superficial punctate keratitis

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Background/aims: The aetiology of Thygeson's superficial punctate keratitis (TSPK) remains elusive. A viral aetiology has been suggested by the absence of bacterial infection and clinical resemblance to other viral keratopathies. We report the results of polymerase chain reaction analysis for the detection of herpes simplex virus (HSV) 1 and 2, herpes zoster virus, varicella zoster virus (VZV) and adenovirus from corneal epithelial samples from patients with active signs and symptoms of TSPK.

Methods: Schirmer strip impressions were taken from the epithelium of eight patients with a known history of TSPK and symptoms and signs of active disease. Three patients were recruited as positive controls (two with herpes simplex keratitis and one with herpes zoster ophthalmicus). Samples from a further three patients acted as negative controls. All 14 samples underwent polymerase chain reaction testing for HSV 1, HSV 2, VZV and adenovirus.

Results: DNA corresponding to the expected viral DNA was amplified from all three positive control samples. The three negative control samples showed no evidence of viral DNA. Similarly, all samples from patients with TSPK showed no evidence of the presence of HSV 1, HSV 2, VZV or adenovirus.

Conclusion: We conclude that HSV, VZV and adenovirus are not present in the epithelium of patients with TSPK. These results are considered in light of existing theories regarding the aetiology and treatment of this condition.

Thygeson's superficial punctate keratitis (TSPK) is characterised by the presence of multiple elevated, white-grey, granular, intraepithelial corneal lesions, located predominantly in the axial cornea and associated with minimal or no conjunctival inflammation.¹ These lesions stain with fluorescein and are associated with symptoms of photophobia and foreign body sensation. They respond favourably to topical corticosteroids, patching or therapeutic soft contact lenses.² However, the course of the disease is one of multiple spontaneous remissions and exacerbations and it can persist for decades.³

It is now more than 50 years since Phillips Thygeson described this condition.¹ Since then it has become a recognised clinical entity in countries across the globe. Despite this the aetiology of TSPK has remained elusive. Thygeson favoured a viral aetiology,⁴ based on: (a) absence of bacteria and other micro-organisms; (b) resistance of the condition to antibiotic treatment; (c) associated scanty mononuclear cell exudates obtained from conjunctival scrapings; and (d) resemblance of the epithelial lesions to those seen in other known viral conditions. However, the resistance to antiviral treatments, the association of TSPK with an increased frequency of HLA-DR3^{5 6} and recent reports of the efficacy of cyclosporin A in achieving long-term resolution all suggest an immunological component to this condition.^{7 8}

To date, investigators using tissue culture and electron microscopy (EM) have failed to demonstrate direct evidence of viral infection in TSPK. Polymerase chain reaction (PCR) analysis allows detection of minute quantities of nucleic acid material from clinical specimens. Furthermore, it is not dependent on replicating viral samples. In this study we report the results of PCR analysis of corneal epithelial samples obtained from individuals with TSPK.

METHODS

Eight patients attending the external eye disease service with an established diagnosis of TSPK were included in this prospective study. The Ethics Committee Approval Board of

the Royal Victoria Eye and Ear Hospital, Dublin, Ireland approved the study, and informed consent was obtained from all participants in the study. For each individual the age, sex, disease duration, number of corneal lesions and presence or absence of symptoms was recorded.

A Schirmer strip impression technique,⁹ was used to obtain cells from the corneal lesions. A lid speculum was inserted. The tear film was allowed to evaporate for 1 min, to maximise epithelial sampling. A sterile Schirmer strip was then pressed directly over the corneal epithelial lesions. The strip was then removed and placed in a vial containing 0.5 ml of phosphate-buffered saline. These vials were then frozen (–70°C) until DNA extraction. Patients were photographed before and after sampling.

Negative control samples were obtained from three patients prior to routine cataract surgery. All negative controls had no evidence of TSPK or previous human simplex virus (HSV), herpes zoster virus or adenoviral infection. Positive control samples were obtained from two patients with large dendritic ulcers. A further control sample was taken from a patient with an active disciform lesion secondary to acute herpes zoster ophthalmicus.

DNA was extracted from all specimens using a viral nucleic acid mini kit (Qiagen, Hilden, Germany). The presence of DNA in each extracted sample was confirmed using an amplification technique for the constitutive gene β -globin. A 110 bp fragment of the β -globin gene was amplified from each sample using the LightCycler Control DNA kit (Roche, Basel, Switzerland). PCR amplifications for HSV, varicella zoster virus (VZV) and adenovirus were carried out using inhouse nested assays, which have been previously described.^{10 11} Distinctions between HSV 1 and HSV 2 were made using a multiplex assay with primers that target the gpD gene (HSV 1) and gpG gene (HSV 2). Positive and negative viral DNA control specimens were included in each PCR assay and detection of amplified product

Abbreviations: TSPK, Thygeson's superficial punctate keratitis; EM, electron microscopy; PCR, polymerase chain reaction; CSA, cyclosporin A

was carried out by gel electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

RESULTS

Patient demographics and clinical characteristics are summarised in table 1. Of eight patients with TSPK, five were female. They ranged in age from 15 to 59 years and had been attending the external eye disease service for between 5 months and 10 years. Of the eight patients with TSPK, seven were symptomatic when samples were taken. Five of these seven patients had between seven and 12 active corneal lesions at the time of sampling (fig 1a–d). All of the subjects tolerated the sampling technique well and reported only minimal discomfort post-sampling.

Adequate DNA was extracted from all samples. Testing of the three positive control samples resulted in amplification of DNA that corresponded to the expected viral DNA (fig 2). No viral DNA was amplified from the three negative controls. All eight samples from patients with TSPK tested negative for HSV 1 and 2. Six samples tested negative for VZV and adenoviral DNA. Sample volume was insufficient for further testing in the remaining two. The results are summarised in table 2.

DISCUSSION

It has always been suspected that TSPK has a viral aetiology, and many investigators have attempted to address and confirm this issue. Braley and Alexandra¹² isolated a single strain of virus that produced lesions in the corneal epithelium of the rabbit; however, subsequent attempts to recover the virus from the corneal lesions failed. Lemp *et al*¹³ in 1974 was able to recover VZV from lesions of a patient with TSPK; however, this has not been repeated. Thygeson⁴ took corneal epithelial scrapings for tissue culture from four patients. Jones¹⁴ also obtained corneal epithelial samples from four patients. Tabbara *et al*¹⁵ obtained corneal epithelial samples from 10 patients. Sundmacher *et al*¹⁶ reported on samples from seven patients. All of these investigators failed to demonstrate viral infection using tissue culture techniques, but had not employed DNA amplification technology.

Multiple investigators have reported on the electron microscopic appearance of samples from TSPK patients. Wakui *et al*¹⁷ described the EM appearance of samples taken from patients with TSPK. Similarly, Sundmacher *et al*¹⁶ reported EM findings of seven patients in 1977. All of these studies failed to reveal viral particles. However, Sundmacher postulated that the virus could be in a latent form within epithelial samples. The altered genetic identity of the cell would stimulate the immune system and cause lymphocytes to surround the affected cells in a hypersensitivity reaction, sensitive clinically to topical corticosteroids.

Darrell⁶ focused attention on the role of the immune system when they reported an association between TSPK and histocompatibility antigen HLA DR3. They postulated that the HLA DR3 antigen alters the immune response of patients with TSPK to exogenous or endogenous viral infections, yielding the prolonged course and the exacerbations and remissions characteristic of the disease. More recently, both Del Castillo *et al*⁸ and Reinhard and Sundmacher⁷ have reported on the efficacy of topical cyclosporin A (CSA) in achieving permanent resolution of the lesions in up to 30% of patients with active TSPK. Del Castillo *et al* postulated that this supported evidence for an immunological condition triggered by a viral infection. Conversely, Reinhard and Sundmacher have postulated that CSA is a less potent suppressor of the immune response than topical corticosteroid treatment. This partial suppression of the immunological response relieves symptoms but leaves enough immunological competence to eliminate the virus. They attribute the low recurrence rate after treatment with CSA to the elimination of a presumed virus.

PCR analysis of samples from patients with TSPK has distinct advantages over tissue culture and EM. Live viral samples are not required and the most minute samples are adequate. Viral DNA can be identified whether the virus is intracellular or extracellular. Hence, PCR has the potential to identify virus in settings where tissue culture and EM studies have previously been negative. Indeed Pavan Langston *et al*⁹ used PCR to demonstrate the presence of varicella zoster DNA in delayed herpes zoster pseudodendrites.

Figure 1 (A) Schirmer sampling technique. (B) Thygeson lesions. (C) Stained with fluorescein low power. (D) Stained with fluorescein high power.

Table 1 Patient demographics and clinical characteristics

Patient details		Symptoms	No. of lesions	Age	Sex	Duration of disease (years)
1	TSPK	Yes	8	16	F	2
2	TSPK	Yes	9	21	F	0.4
3	TSPK	Yes	12	33	M	10
4	TSPK	Yes	2	21	F	1
5	TSPK	No	2	33	F	2
6	TSPK	Yes	3	30	M	2
7	TSPK	Yes	12	57	F	2.5
8	TSPK	Yes	7	15	M	9
9	+Con HSV-1			59	M	
10	+Con VZV			56	M	
11	+Con HSV-1			76	F	
12	-Con			75	M	
13	-Con			68	F	
14	-Con			59	M	

Con, control.

Recent literature has examined three molecular methods that evaluate herpes simplex detection. In this paper PCR had a specificity of 74.4% and a positive predictive value of 63.6%.¹⁸ The detection rates for other viral pathogens is unknown. We used the same Schirmer strip impression technique as described by Pavan Langston *et al*⁹ in order to obtain corneal epithelial cells adjacent to and overlying active staining lesions. This technique is relatively non-invasive (when compared with taking corneal epithelial scrapings) and our patients reported little or no discomfort after sample taking. However, potential sample limitations pertaining to the depth of sampling exist by this method, as samples are from the superficial epithelium. It is possible that deeper, subepithelial viral infection could be missed. The timing in the clinical course of the disease entity when sampling is procured could potentially affect the viral yield. In this study all patients had evidence of active disease at the time of sampling. Despite this we consider this to be an appropriate method as TSPK has been unambiguously described as an epithelial condition with no subepithelial involvement.^{1-6,14} Epithelial oedema can give rise to what appears to be a faint opacification of the anterior stroma. However, retro-illumination of these lesions will reveal that the stroma itself is uninvolved.¹⁴ This is in contrast to adenoviral lesions in which the subepithelial component is an infiltration rather than oedema alone.

A number of candidate viruses have been implicated as potential causes of TSPK, including HSV, herpes zoster virus, adenovirus and Epstein-Barr virus due to similar clinical presentations, but often with differing clinical courses. TSPK initially presents with small, round, branched epithelial lesions leading to an inappropriate diagnosis of herpes simplex keratitis.⁶ These lesions are frequently treated with antivirals in the initial stages. Reports on the efficacy of the antiviral treatment have varied.^{1,19} Both HSV and VSV can have long latent periods and recur over long periods of time in common with TSPK. Adenovirus gives rise to a clinically distinct condition. However, the presentation and clinical course are entirely different to patients suffering with TSPK. The corneal epithelial lesions can be similar but they have a subepithelial component and the course of the disease is not prolonged. Epstein-Barr virus can give rise to a punctate keratitis, which is usually mild and self-limiting and has a distinctive appearance with some subepithelial involvement. TSPK has also been reported after documented chlamydial follicular conjunctivi-

1 2 3 4-11 12 13 14 Lane

- 1 Molecular weight markers.
- 2 HSV-1 + control
- 3 HSV-2 + control
- 4-11 TSPK samples
- 12 Clinical + control (HSK)
- 13 Clinical - control
- 14 Clinical + control (HZO)

Figure 2 Photograph of gel electrophoresis plate demonstrating the amplified product of the polymerase chain reaction test for HSV 1 and 2. There is a molecular weight marker at each side of the gel (broad white arrow). Lanes 2 and 3 contain positive controls for HSV 1 and HSV 2 respectively (broad arrows). Two positive specimens for HSV 1 are shown in lanes 12 and 14 (black arrows). HSK, herpes simplex keratitis; HZO, herpes zoster ophthalmicus.

tis.²⁰ Staphylococcal hypersensitivity has also caused diagnostic confusion.

In this study, the Schirmer impression strips were placed in 0.5 ml of phosphate-buffered saline. This volume was chosen in order to avoid excessive dilution of the sample material. As each PCR assay requires at least 0.1 ml of PBS we were limited in the number of viruses that we could test for. We chose HSV 1, HSV 2 and VZV as the issue of antiviral treatment has never been fully resolved. Trifluorothymidine has been reported as efficacious¹⁹ and this condition is frequently treated with topical aciclovir. We chose adenovirus as there have been occasional reports in the literature of adenoviral keratoconjunctivitis that

Table 2 Results of polymerase chain reaction analysis of epithelial samples

Patient	Patient details	HSV	VSV	Adenovirus
1	TSPK	N	N	N
2	TSPK	N	N/A	N/A
3	TSPK	N	N/A	N/A
4	TSPK	N	N	N
5	TSPK	N	N	N
6	TSPK	N	N	N
7	TSPK	N	N	N
8	TSPK	N	N	N
9	+Con HSV 1	+POS	N	N
10	+Con VSV	N	+POS	N
11	+Con HSV 1	+POS	N	N
12	-Con	N	N	N
13	-Con	N	N	N
14	-Con	N	N	N

+POS, positive; N, negative; Con, control; N/A, not applicable.

has persisted over years with multiple remissions and exacerbations.²¹

Our results demonstrate conclusively that none of our candidate viruses (HSV 1, HSV 2, VSV and adenovirus) are present in the epithelium of patients with TSPK. This indicates that the condition is not the result of an immunological response to a latent or intracellular epithelial infection with one of these viruses. It is possible that one of the other aforementioned candidate viruses could be present in the epithelium. Alternatively it is possible that the lesions are in the anterior stroma. Such an explanation would require a fundamental re-evaluation of the description of this condition. A recent case report has, however, described one case of TSPK that resolved after photorefractive keratectomy for myopia.²²

TSPK was described over 50 years ago. Worldwide, it is a chronic condition, the true incidence of which is probably underestimated. Despite this, progress in elucidating the aetiology of the condition has been slow. To our knowledge this is the first study to apply PCR technology to TSPK. This is also the first study to use Schirmer strip impressions for taking corneal epithelial samples in TSPK. Both of these techniques have further potential and could be used to systematically exclude other potential pathogens. If epithelial studies prove negative it may be justifiable to subject a small number of patients to deeper corneal scrapings for analysis of anterior stromal samples. Such an analysis may finally give a definitive answer regarding the presence of viruses in this condition.

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