# DETECTION OF VARICELLA-ZOSTER VIRUS DNA IN OCULAR SAMPLES FROM PATIENTS WITH UVEITIS BUT NO CUTANEOUS ERUPTION

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## SUMMARY

**Herpes zoster ophthalmicus is a well-recognised cause of intraocular inflammation, which may become recurrent or chronic after the acute phase has elapsed. Although it commonly presents with the typical rash, cases of ocular zoster with no cutaneous eruption have been well documented. We present two patients with unilateral anterior uveitis complicated by cataract, in whom molecular techniques based on the polymerase chain reaction detected varicella-zoster virus DNA in intraocular material obtained during cataract surgery. Neither patient gave a history of cutaneous eruption.**

Herpes zoster ophthalmicus (HZO) is a common disease caused by the reactivation of latent infection by the varicella-zoster virus (VZV).[1–4] An important complication is anterior uveitis, which develops in 43–53% of patients.[4–6] Its onset may be delayed for months after the disappearance of the rash[1,4,5] and can vary in severity from mild to severe.[3,4] Keratic precipitates, anterior and posterior synechiae are common,[2,7] and iris atrophy, although usually a late manifestation, may occasionally be present during the acute stage.[1,7] This is characterised by sectoral loss of iris pigment epithelium and migration of pigment into the overlying stroma and is thought to be secondary to an occlusive vasculitis.[3,4,8] Posterior subcapsular cataract may develop as a result of chronic uveitis or prolonged use of topical steroids.[1,4] Secondary glaucoma, seen in 15–43% of patients with anterior uveitis due to HZO,[5] is thought to be due to a combination of several mechanisms including trabeculitis and plugging of the trabecular meshwork with debris and cells, posterior synechiae causing pupillary block and anterior synechiae causing angle closure.[1,4]

While the clinical diagnosis of HZO is relatively easy when the typical cutaneous eruption is present, patients with intraocular inflammation can be a diagnostic dilemma when there is no history of rash or evidence of skin scarring.[1,4,9] In the past, the availability of only small volumes of ocular fluid and the high prevalence of serum antibodies to herpes viruses in the general population has meant that making a specific diagnosis in such circumstances has been difficult. Recently, molecular techniques based on the polymerase chain reaction (PCR) have been applied successfully to a variety of ocular samples to identify viruses in cases of intraocular inflammation.[10–16] The PCR has proven advantages as a diagnostic aid in view of its specificity for amplification of specific genomic sequences from an infectious agent, requiring only minute specimens and giving results within several hours.

We present two patients with unilateral uveitis of unknown aetiology and no history of cutaneous eruption. In both patients VZV DNA was isolated from intraocular samples obtained during cataract surgery, after amplification by the PCR.

## PATIENTS AND METHODS

### *Case 1*

A 62-year-old healthy, immunocompetent, Caucasian male initially presented to the Accident & Emergency Department of the Birmingham and Midland Eye Hospital in September 1990, complaining of a red, painful left eye associated with blurred vision of 9 days' duration. His past ocular history was unremarkable. He was found to have a left anterior uveitis with 'mutton fat' keratic precipitates, dense flare and cells, posterior synechiae, an intraocular pressure of 22 mmHg and a visual acuity reduced to 6/18. The other eye was normal. He improved on topical steroids and was discharged after 2 months.

He re-presented 6 days later with a relapse of the uveitis. At that visit, sectoral iris atrophy was noted. Routine uveitis investigations were negative except for an elevated plasma viscosity of 1.88 cP (normal range: 1.50–1.72 cP). The anterior uveitis became chronic in nature and a posterior subcapsular cataract developed.

In December 1992 he underwent a left extracapsular

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cataract extraction which was complicated by vitreous loss, although a posterior chamber intraocular lens (IOL) was implanted. During the operation, aqueous humour, lens nucleus and vitreous humour were obtained.

His chronic uveitis persisted after his cataract extraction and while his immediate post-operative acuity was 6/18, it deteriorated to 6/36 in June 1993 due to cystoid macular oedema (CMO). Although the CMO resolved after a periocular injection of depot steroid, his visual acuity remains at 6/18 due to posterior lens capsular thickening. In view of the sector iris atrophy, there was a clinical suspicion of VZV being the cause of this patient's chronic unilateral uveitis.

### *Case 2*

A 55-year-old healthy, immunocompetent, Caucasian male has been attending the Birmingham and Midland Eye Hospital since 1976 with a recurrent left anterior uveitis and secondary glaucoma. His other eye has always been quiet. Routine uveitis investigations have been normal except for a positive Mantoux test in 1976. As a result of his recurrent inflammation he developed a posterior subcapsular cataract.

In January 1993 he underwent an uneventful extracapsular cataract extraction and a heparin surface-modified posterior chamber IOL was implanted. During the operation, aqueous humour and lens nucleus were obtained.

Post-operatively, his visual acuity improved to 6/6 and he still continues on topical steroid and beta-blocker. As this patient's hypertensive anterior uveitis had always been unilateral over 17 years, there was a clinical suspicion of herpes simplex virus-1 (HSV-1) being the aetiological agent.

### *Method for the Detection of Herpesviral DNA in Ocular Samples*

All ocular samples were taken under sterile conditions and then stored at −50 °C. Prior to analysis, the aqueous and vitreous humour samples were boiled for 10 minutes then rapidly cooled on ice. The lens nucleus samples were dissolved in buffer containing sodium dodecyl sulphate and proteinase K, boiled for 10 minutes, then incubated overnight at 37 °C.

The nested PCR method used for the detection of viral DNA sequences from VZV gene 29, HSV-1 gD and cytomegalovirus (CMV) gB in ocular fluids has been reported previously[15,16] and was applied to the ocular samples from the patients described here. The method utilises oligonucleotide primers adapted from those described by Mahalingham *et al.*,[17] Aurelius *et al.*[18] and Darlington *et al.*[19] The first-round reaction mix contained 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1 unit of *Taq* polymerase (Perkin Elmer Cetus, ILS Ltd, London), 200 µM of each dNTP, 100 ng of each 'outer' primer and 1 µl of aqueous or vitreous fluid. After an initial 5 minute denaturation at 95 °C, 35 cycles of 95 °C for 2 minutes, 50–60 °C for 2 minutes and 72 °C for 1 minute were carried out, followed by a 7 minute extension at 72 °C using an automated thermal cycler. The reaction conditions for the second round of PCR were as for the first except that the mix contained 100 ng of each 'inner' primer pair and 1 µl of the first-round product. For detection of the second-round product, 25 cycles were sufficient. The first- and second-round products were analysed by electrophoresis using a 2% agarose gel and were visualised at 302 nm after staining with ethidium bromide. The extracted material from lens nucleus was treated in a similar way and 10-fold dilutions were added to the PCR reaction.

Aqueous samples were also obtained at cataract extraction from 20 control patients with senile cataract and 20 patients with Fuchs' heterochromic cyclitis who acted as a disease control group.[20]

## RESULTS

In the aqueous samples no VZV, HSV-1 or CMV DNA sequences could be detected in either case. In the vitreous samples VZV DNA but not HSV-1 or CMV DNA was detected in case 1; no herpesviral DNA sequences were found in case 2. In the lens nucleus VZV DNA but not HSV-1 or CMV DNA was detected in case 2; no herpesviral DNA sequences were found in case 1.

No herpesviral DNA sequences were found in aqueous samples from either control group.

## DISCUSSION

Although viruses have been implicated as causative agents in various forms of uveitis,[3,10,15,16,21–29] the clinical diagnosis can occasionally be difficult, particularly in atypical cases. Confirmation of a viral aetiology using laboratory techniques usually is unsatisfactory as demonstration of antiviral antibodies in the serum does not confirm end-organ disease. Increased concentration of specific antibodies in aqueous humour and vitreous is also an indirect sign of viral infection. Culturing herpes viruses from intraocular fluids is difficult, requires relatively large amounts of sample, and may take a considerable time until the result is known. Recent work by a variety of authors has shown the value of applying molecular techniques based on the PCR to the detection of herpes viruses in ocular samples.[10–16] Inherent to these techniques is their rapidity, sensitivity and specificity when compared with previous laboratory-based methods.

The molecular technique used detected fragments of VZV DNA and as such does not indicate actively replicating virus. Treatment of the samples does not differentiate between cell-associated viral DNA and 'free' virus. Nevertheless, fragments of unencapsulated viral DNA are unlikely to persist without being degraded, so our findings almost certainly reflect the presence of VZV, latent or otherwise. Also, as herpesviral DNA was not present in the controls, it is highly probable that VZV was the causative agent of the intraocular inflammation. In the future, identification of primers specific for messenger RNA sequences for proteins involved in active viral replication or for latency-associated proteins would allow

differentiation between active and latent viral infections by the PCR technique, which is not possible now.

The first case of ophthalmic herpes zoster without cutaneous eruption was described by Von Hoffmann in 1879.[9] Further reported cases have presented with various ocular manifestations including ophthalmic neuralgia, dendritiform keratopathy, disciform keratitis, uveitis and secondary glaucoma, but no cutaneous vesicles at any time during the process of the disease.[1,4,9] Some authors have expressed the view that the presence of sector iris atrophy confirms that the patient has at some stage suffered from HZO even though the other signs of the disease such as skin scarring and a typical history may be absent.[3] A well recognised manifestation of ocular herpes zoster infection without cutaneous eruption is acute retinal necrosis (ARN) where VZV has been shown to be the causative agent. VZV DNA has been detected in aqueous and vitreous samples using the PCR,[12] and identified in the choroid and choriocapillaris.[13] Although ARN is described in immunocompromised individuals, such as those suffering with the acquired immunodeficiency syndrome (AIDS), it is often found in immunocompetent patients. VZV has also been implicated in the progressive outer retinal necrosis (PORN) syndrome seen in AIDS patients, but in a number of these cases there was an association with episodes of zoster dermatitis.[28,29]

As our cases were not associated with cutaneous eruption, this would imply that the uveitis resulted from a reactivation of virus deposited at the time of the original varicella attack. It is probable that after a period of latency in the sensory ganglia, the neurotropic virus reactivates and moves along the peripheral branches of the affected nerves to involve the eye. Recently, Dua *et al.*[30] described two clinical forms of anterior uveitis: an 'iris pigment epithelitis' and an 'iris vasculitis'. The anterior uveitis resulting from HSV-1 is thought to be an iris pigment epithelitis, whereas that due to VZV probably results from an iris vasculitis. The iris stromal atrophy seen in case 1 was identical to that found in association with classic herpes zoster ophthalmicus. This type of clinical picture is invariably unilateral, in contrast to ARN where bilateral involvement is common. In view of the bilaterality, it has been proposed that ARN may result from a haematogenous route of viral dissemination rather than from transport down a bilateral neural pathway from the central nervous system.[31] As yet, the factors responsible for causing reactivation of the virus are unknown, although the local and/or general immunological environment may have a role to play. As VZV DNA was found in lens nucleus in case 2, sequestration of virus to lens or other intraocular structures could occur with reactivation triggered by as yet unknown local factors.

Careful collection of intraocular samples and standardisation of the PCR steps is very important to eliminate contamination, which is a danger for such a sensitive technique. To overcome possible contamination, all samples were taken under sterile conditions in the operating theatre and immediately transferred to sterile containers and stored at −50 °C. The nested PCR is a powerful and sensitive technique and using the methods described in the paper was able to detect single copies of plasmid DNA for CMV. The detection sensitivity of HSV-1 was equivalent to 0.01 infectious units and for VZV was equivalent to 0.01 infected cells. With this level of sensitivity, a false positive or mistyping may occur when the majority of the molecules to be detected arise from an exogenous source rather than from the sample itself or when there is 'carryover' of large numbers of amplifiable molecules from one amplification to the next. To control contamination, the physical transfer of DNA between amplified samples and between positive and negative experimental controls must be avoided. In the laboratory, the guidelines laid down by Kwok and Higuchi[32] to avoid such problems were adhered to strictly.

The PCR has been used for the detection of viral DNA in cases of posterior segment inflammatory disease, such as ARN and retinitis in immunocompetent and immunocompromised patients.[11–16] In these cases, early treatment with the most appropriate antiviral agent may prevent potentially vision-threatening complications. It is only recently that the PCR has been used in establishing a viral aetiology for cases of anterior uveitis.[10]

An accurate diagnosis of herpetic intraocular inflammation is important as long-term control of the inflammation may be required. Clinicians will also be alerted to the possibility of chronicity or recurrence.

The PCR is a quick, sensitive and specific method for detecting viral DNA that requires minimal amounts of intraocular fluid. It appears to be a useful tool in establishing the aetiological diagnosis in cases of intraocular inflammation due to suspected viral infection.

Key words: Cataract, DNA, Polymerase chain reaction, Uveitis, Varicella zoster virus.

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