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SHORT REPORT

Absence of Herpesvirus DNA in Aqueous Humor from Asymptomatic Subjects

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Purpose: To assess herpesvirus DNA detection in aqueous humor from a cohort of asymptomatic Scandinavian patients undergoing elective cataract surgery.

Patients and Methods: Prospective case series. Aqueous samples were obtained from 30 patients undergoing elective cataract surgery. Polymerase chain reaction (PCR) analysis for herpes simplex virus 1 (HSV1), herpes simplex virus 2 (HSV2), cytomegalovirus (CMV), Epstein Barr virus (EBV) was performed. Toxoplasma was added to the analysis due to its role as pathogen with ocular latency.

Results: Mean age of participants was 75.3 years. Sixteen subjects (53%) had ocular comorbidities. Five subjects (17%) had endothelial dysfunction without known hereditary pattern. None of the samples were positive for herpesviruses or toxoplasma.

Conclusion: None of the aqueous samples were positive, suggesting shedding does not frequently occur in the aqueous humor of asymptomatic patients.

Keywords: shedding, aqueous humor, herpes simplex virus, varicella zoster virus

Introduction

Herpesvirus are a group of ubiquitous large, enveloped DNA viruses that can infect numerous species. Ancestors of Homo sapiens were presumably infected from closely related primates and since then, a common evolution between host and virus has continued.[1–3] In modern times, herpes viruses accompany us as latent stowaways with the ability to cause severe disease if the opportunity is given. Varicella zoster virus (VZV) and herpes simplex virus 1 (HSV1) and 2 (HSV2) are alpha herpes viruses with common tropism for the neuronal system including the establishment of latency in sensory or autonomic ganglia. In the case of ocular disease, latency is localized in the trigeminal ganglion or possibly in the ciliary ganglion and cornea.[4–6] It has been shown previously that HSV1 has the ability to shed virus regularly in the tears of asymptomatic patients.[7,8] VZV has by far fewer symptomatic recurrences over time. Despite this, shedding of viral genome has been detected in tears of asymptomatic individuals.[9]

Quantitative Real-time Polymerase Chain Reaction (qPCR) is widely used as an eminent tool for detecting virus genome. It is of importance in diagnostics of uveitis as detecting an infectious cause of inflammation has major impact on choice of treatment and visual outcome. Sampling is preferably taken from the intraocular fluids, most commonly aqueous humor from the anterior chamber of the eye.[10]

To draw conclusions on a positive test result, we need to ascertain that there is no asymptomatic shedding of virus in the aqueous humor. In our study, we sampled aqueous humor from 30 asymptomatic patients in conjunction with cataract surgery for detection of herpes viruses by qPCR analysis.

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Patients and Methods

Participants in the study were recruited at the Department of Ophthalmology at Halland Hospital in Halmstad between March 2016 and March 2017. Folders with information were sent by mail to patients who were planned for elective cataract surgery at the clinic. Informed consent was obtained from each patient at the preoperative visit. The study was conducted in accordance with the declaration of Helsinki and approved by the Swedish Ethical Review Authority. Other ocular disease was not considered an exclusion criterion. Sampling was performed intraoperatively with a 30-gauge needle, aspirating 100–200 microliters of aqueous humor from the anterior chamber under sterile conditions. Specimens were immediately frozen and stored at −20°C. Analysis took place at the Clinical Virology unit of the Microbiology laboratory at Sahlgrenska University Hospital, Gothenburg, Sweden. DNA was extracted in a MagNa Pure LC robot (Roche Diagnostics, Mannheim, Germany) using the MagNa Pure DNA Isolation Kit according to manufacturer´s instructions. Real-time qPCR for HSV1, HSV2, VZV, cytomegalovirus (CMV), Epstein Barr virus (EBV) and toxoplasma was used as described previously.[11,12] Toxoplasma was included due to its capability of latency and pathogen of ocular disease.

Results

There were no complications during sampling of aqueous humor in conjunction with cataract surgery. Thirty aqueous humor samples were collected and in total, 180 analyses were performed. None of the aqueous humor samples were positive for HSV1, HSV2, CMV, EBV, VZV or toxoplasma.

In the patient cohort, mean age was 75.3 (SD ± 6.60) years. There were 20 women (mean age 75.9 ±7.15) and 10 men (mean age 74.2 ±5.49). Sixteen (53%) had ocular comorbidity. Three (10%) were diagnosed previously with age-related macular degeneration (AMD). Six (20%) had glaucoma with medication and one had diabetic retinopathy. One patient had recurrent anterior uveitis and scleritis (untreated and asymptomatic three months prior to surgery). None of the participants in this study used systemic or topical steroids three months before surgery. We chose to include 5 patients (17%) with cornea guttata because of the association between CMV reactivation and endotheliitis.[13]

Two patients developed minor complications in the postoperative period, none of them had ocular comorbidities. One had late onset corneal edema and one developed prolonged iritis.

Discussion

In our material of cataract patients in Sweden, none of the patients had herpes virus DNA or toxoplasma in the aqueous humor at the time of surgery. This is in keeping with most previous reports[10,14–17] with few exceptions.[18] (Table 1) These data taken together, we reach a higher probability to discard the hypothesis of at least frequent shedding in aqueous humor in asymptomatic eyes, which in turn may strengthen the diagnostic importance of positive results from this body fluid. In contrast, there is documented shedding in the tear film, measured by qPCR.[8,19,20] Kaufman et al presented 49 out

Table 1 Previous Publications with Herpes Virus PCR-Analysis of Aqueous Humor in Asymptomatic Patients Taken in Conjunction with Intraocular Surgery

	PCR	Sample Size	Results
Rothova 2008[10]	HSV1, HSV2, VZV, CMV, (toxoplasma)	20	Negative
Yamamoto 1996[14]	HSV, VZV, CMV	10	Negative
Laaks 2015[15]	HSV1, HSV2, VZV CMV, EBV, HHV-6	57	Negative
Pendergast 2000[16]	HSV1, HSV2, VZV, CMV, EBV	35	Negative
Kerochana 2018[17]	HSV1, HSV2, VZV, CMV, EBV	66	Negative
Cimino 2013[18]	HSV, VZV, CMV, (rubella virus)	27	Positive: 1 CMV, 1 HSV

Abbreviations: CMV, cytomegalovirus; EBV, Epstein Barr virus; HHV-6, human herpes virus 6; HSV, herpes simplex virus; PCR, polymerase chain reaction; VZV, varicella zoster virus.

of 50 subjects with 74% seropositivity for HSV IgG to shed HSV1 in tears at least once during the course of 30 days. Surprisingly, even seronegative subjects exhibited shedding.[7] Ramchandani et al followed eight seropositive individuals for 291 days and found HSV1 DNA to be detected by qPCR in 26.5% of days. In 7.6% of days this could also be confirmed by virus culture suggesting infectious virus.[8]

To exclude any possible shedding of viral DNA in the aqueous humor, a repeated daily aqueous tap of the same patient would be necessary. Nevertheless, this investigation would not be considered ethical in asymptomatic subjects.

Five subjects in our study had corneal endothelial dysfunction with signs of cornea guttata at slit-lamp examination. These patients denied presence of cornea dysfunction in relatives (such as autosomal dominant Fuchs endothelial dystrophy). As CMV endotheliitis can give rise to endothelial dysfunction, mostly documented in Asian subjects with positive qPCR,[21] we found it interesting to include CMV in the analysis. However, these samples were negative as well. Still, there is a possibility of a positive result using repeated sampling or combined analysis with antibody-index and PCR.

Conclusion

Herpes viruses in humans seem to have found a way of latency and effective shedding through body fluids excreted externally, for example through tears and saliva. This enables spread to other hosts. However, internal fluids like aqueous humor appear to be free of virus when drawn from asymptomatic subjects, similar to what has been found in cerebrospinal fluid.[22]

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Disclosure

The authors report no conflict of interest in this work.

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