Real-Time Polymerase Chain Reaction for Diagnosing Acyclovir-Resistant Herpetic Keratitis Based on Changes in Viral DNA Copy Number Before and After Treatment

Acyclovir (ACV) is a specific antiviral agent that is active against herpes simplex virus (HSV) and anti–varicella-zoster virus agent for herpetic infection; topical ACV ointment, 3%, has been the treatment of choice for HSV infection in Japan. Based on the widespread use of ACV, in some cases of recurrent herpetic keratitis, the disease was refractory to topical ACV treatment, which was previously reported as ACV-resistant herpetic keratitis. Conventionally, definitive diagnosis of an ACV-resistant HSV keratitis requires a laboratory examination of viral cultures following in vitro drug sensitivity testing, which is generally difficult and complicated because of the low rates of replication of these viruses and the small number of ocular samples. A rapid, simple, and accurate method of diagnosing drug-resistant herpetic infection remains to be established. Real-time polymerase chain reaction (PCR) is a highly sensitive method for the detection and quantification of pathogens. We report 4 cases of ACV-resistant HSV keratitis using real-time PCR analysis; these cases were diagnosed based on changes in the viral DNA copy numbers before and after ACV treatment.

Report of Cases. Case 1 is representative of the 4 cases. The patient was a 37-year-old man with a history of herpetic keratitis in the left eye that was diagnosed at another clinic. Initially, the herpetic keratitis resolved with periodic treatment using ACV ointment and a topical steroid. He had reported a foreign-body sensation and redness in his left eye and periodically received topical antibiotics and a steroid for 1 month. Because the symptoms gradually worsened despite treatment, he was referred to our clinic. At his first examination, his affected eye had a best-corrected VA of 20/1000 and an intraocular pressure of 15 mm Hg. A slitlamp examination revealed epithelial erosion in the central cornea that manifested as geographic lesions with corneal infiltrates, moderate ciliary injection, and no anterior chamber inflammation (day 0) (Figure, A). Using a Cochet-Bonnet esthesiometer, we determined that the sensitivity of the left cornea decreased to 10 mm in the left eye compared with 60 mm in the right eye. The right eye was normal. A wide corneal scraping was performed for cytopathologic examination and culturing to detect any pathogenic microorganisms such as bacteria, fungi, or Acanthamoeba, and real-time PCR analysis was performed to detect human herpesviruses (HSV type 1 or 2, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6, 7, and 8). Light microscopy failed to identify any pathogens. The PCR results were positive only for HSV-1 DNA (8.7 × 10^7 copies/sample) (Figure, E). The culture results were negative for any pathogens. Based on the positive PCR results for HSV-1 DNA, the patient was suspected to have HSV keratitis and was treated with topical ACV ointment, 3%, 5 times a day and topical levofloxacin, 0.5%, 3 times a day for 2 weeks. However, the geographic lesions did not heal (day 14) (Figure, B). A second epithelial scraping was performed for real-time PCR analysis and cytopathologic examination and culturing, which resulted in detection of only HSV-1 DNA (1.2 × 10^6 copies/sample) (Figure, E) from the diseased lesions without other pathogens. Because ACV is a specific anti-HSV and anti–varicella-zoster virus agent, it usually is effective against herpetic keratitis, and, simultaneously, it can decrease HSV copy numbers compared with pretreatment (data not shown). However, the HSV viral load in this case did not decrease as a result of ACV treatment, and the only detected pathogen was HSV. Because these findings indicated that the HSV detected in these lesions might be an ACV-resistant strain, we substituted topical trifluorothymidine (TFT) solution, 1%, prepared from TFT, which was reported to be effective against ACV-resistant HSV keratitis. The levels of ocular pain and photophobia gradually decreased, and the lesions slowly healed by day 28 (Figure, C). Real-time PCR of the samples obtained from epithelial scraping 14 days after the start of TFT therapy did not detect HSV-1 DNA (Figure, E), and by day 42, the keratitis resolved with corneal scarring (Figure, D).

Our Table shows the clinical characteristics and the HSV DNA copy numbers of 4 eyes from 4 cases through the therapeutic clinical course. In the current case series, the patients in cases 1, 2, and 3, who had been clinically diagnosed with herpetic keratitis (cases 1 and 2) or keratouveitis (case 3), had not undergone a herpetic PCR examination but received antiviral therapy and/or a topical steroid periodically. The patients in cases 3 and 4 had undergone penetrating keratoplasty and were treated with topical steroid constantly. For these patients, the microbiologic examinations included histologic examinations, cultures, and real-time PCR, and only a high number of copies of HSV DNA were detected from their lesions (no bacteria, fungi, Acanthamoeba, or other human herpesviruses). All patients received ACV antiviral therapy; however, the clinical appearance did not improve. The high number of copies after treatment suggested that the HSV was an ACV-resistant strain. Because of this, we treated all patients with TFT, and the keratitis resolved with a significant decrease in HSV copy numbers.

Comment. To the best of our knowledge, this is the first case series to report ACV-resistant HSV keratitis diagnosed by changes in the viral DNA copy numbers before and after ACV treatment using real-time
PCR analysis. Because the conventional determination of an ACV-resistant HSV strain is technically difficult and requires experience, complex skills, and time to obtain results, application of real-time PCR to drug-resistant HSV keratitis is preferable because of its simplicity, high sensitivity, and rapid results. Furthermore, for differential diagnoses, PCR can identify other pathogens simultaneously from limited volumes of ocular samples, and this method of estimation is available using routine HSV detection primers with no need for additional examinations.

The characteristics of our 4 patients were consistent with the characteristics of patients described in previous papers with regard to the details of the therapeutic history, steroid use, immunocompetency, slowly progressive disease, and refractoriness to ACV therapy.1-3 The patients in cases 2, 3, and 4 had no previous independent therapeutic experience with ACV ointment; however, long-term use of ACV ointment in conjunction with a topical steroid agent may have induced the ACV-resistant HSV infection in their corneas. This resistance could have resulted from the local immune status based on irregular and/or inadequate use of a topical steroid or an ACV ointment.1,2 The clinical improvements associated with TFT validated our real-time PCR results for diagnosing ACV-resistant HSV keratitis.

Although this method can be used to diagnose ACV-resistant keratitis, it cannot identify ACV-resistant strains, which necessi-
tates culture and drug-sensitivity testing. However, this method of estimation by real-time PCR is especially helpful when the anti-herpetic agent is ineffective and when clinical signs and risk factors of herpetic infection are present, along with the detection of herpetic DNA, and would allow ophthalmologists to make a more rapid and accurate diagnosis of ACV-resistant herpetic keratitis.

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The adult Taenia solium tapeworm remains confined to the small intestines; however, in the larval stage known as cysticercosis cellulose, this tapeworm has been identified in many other organs, including the eye. The juvenile strobilate tapeworm typically remains confined to the intestines; however, Bamrungphol et al report an extraintestinal manifestation in the spinal cord. We report 2 cases of live juvenile strobilate tapeworm, Taenia solium, seen in the anterior chamber of the eye.

Report of Cases. Case 1. A 48-year-old man living in the Himalayan foothills of North India presented with redness, pain, and progressive loss of vision in the left eye for over 4 months. His best-corrected visual acuity was 6/6 in the right eye and light perception in the left eye.