Herpes Simplex DNA in Tears of Atypical Dendritic Keratitis and Multiple Punctate Subepithelial Stromal Opacity: A Case Report

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Purpose: To report an atypical presentation of herpes simplex virus (HSV) keratitis followed up using expression levels of HSV DNA in tears.

Methods: A 22-year-old Japanese woman with hyperemia and foreign body sensation in her left eye was diagnosed with atypical dendritic keratitis. A slit-lamp examination at presentation indicated the presence of a rush of dendritic lesions with a sparse branching pattern and poor development of terminal bulbs; follicular conjunctivitis was also observed. Positivity for house-dust-mite- and cedar pollen-specific IgE antibodies in her serum indicated atopic diathesis. The HSV DNA levels in her tears were measured by a real-time polymerase chain reaction.

Results: At the initial visit, the HSV DNA levels in tears were 6.4 \times 10^6 copies/sample in the right eye and 1.6 \times 10^5 copies/sample in the left eye. The keratitis improved after treatment with topical acyclovir ointment, 5 times a day for 7 days, and systemic valacyclovir 1000 mg/d for 5 days. Multiple punctate subepithelial opacities developed in her left eye on day 7, with undetectable HSV DNA in tears, bilaterally.

Conclusions: We have successfully monitored the HSV DNA levels in tears using quantitative real-time polymerase chain reaction in HSV keratitis where the corneal findings progressed from atypical dendritic keratitis to multiple punctate corneal subepithelial opacities during the treatment period.

Real-Time PCR for HSV DNA Levels in Tears

Tear samples were obtained by the Schirmer test that was performed without topical anesthesia using Schirmer test papers (tear production measuring strips; Ayumi Pharmaceutical Co, Tokyo, Japan). The test papers were placed in the lower conjunctival fornix of both eyes for 3 minutes and then removed. The Schirmer test paper with a 5-mm tip was used for the HSV DNA assay. Real-time PCR was performed using the GYBR Green method. Primers specific to the DNA polymerase region of HSV-1 and HSV-2, with forward and reverse primers for HSV-1 and HSV-2, were designed. The PCR was performed with a LightCycler 480 II (Roche Diagnostics, Mannheim, Germany) using the SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA). The PCR reaction mixture consisted of 50 ng of DNA, 5 mM MgCl₂, 0.2 mM of each primer, 0.5 mM of each dNTP, 400 nM of SYBR Green, and 2× LightCycler 480 II Master Mix in a final volume of 20 μL. The PCR cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. The specificity of the amplification was confirmed by melt curve analysis and by melting curve analysis of the specific primer pairs.

METHODS AND CASE PRESENTATION

Corneal infections are commonly caused by herpes simplex virus (HSV) type 1 (HSV-1) and occasionally by HSV type 2 (HSV-2). Typical herpes simplex virus keratitis (HSK) is divided into 3 subgroups: epithelial keratitis, which includes dendritic and geographic keratitis; stromal keratitis, which includes disciform and necrotizing keratitis; and endotheliitis. However, HSK related to HSV reactivation shows atypical corneal lesions depending on the ocular surface viral load, associated corneal disorders, and the patients' immune status including atopic dermatitis and immunodeficiency. These atypical and varied clinical presentations challenge the therapeutic decision-making process for the appropriate and adequate use of acyclovir and corticosteroids.

Real-time polymerase chain reaction (PCR) is a clinical laboratory test that measures HSV viral load in ocular surface specimens, including tears. Real-time PCR has demonstrated utility in diagnosing HSK and has been proven to be effective in the medical treatment of HSK. Therefore, we speculate that the ocular surface viral load may serve as a clinical marker for deciding and monitoring the appropriate treatment strategy for HSK. This case report presents the results of monitoring HSV DNA in tears before, during, and after medical therapy for atypical dendritic keratitis in a patient who subsequently developed multiple punctate subepithelial stromal opacities.
primer sequence 5′-CAT CAC CGA CCC GGA GAG GGA C-3′ and reverse primer sequence 5′-GGG CCA GGC GCT TGT TGG TGT A-3′ were used. DNA from each Schirmer test paper was harvested using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and was used for PCR. Real-time PCR was performed using a StepOnePlus real-time PCR system (Life Technologies, Japan) and comparative threshold (Ct) values were obtained.

Case Report

A 22-year-old Japanese woman who wore daily disposable contact lenses presented to the Nihon University Itabashi Hospital, Tokyo, Japan, with a 7-day history of eye pain and hyperemia in her left eye. Her symptoms started the morning after she went to bed wearing her contact lens, for which she visited a local eye clinic and was diagnosed with conjunctivitis (day 0). Her conjunctivitis was treated with topical 0.3% ofloxacin ophthalmic ointment and 0.5% levofloxacin ophthalmic solution for 7 days. However, her symptoms persisted. Seven days after the initial onset, she was reexamined and diagnosed with HSK at the local eye clinic. She did not have a medical history of herpes simplex virus ocular infection. She was then referred to the Nihon University Itabashi Hospital for further management (day 7).

At presentation (day 7), her best-corrected visual acuity in the affected eye was 12/20 (decimal visual acuity), and the
intraocular pressure was 22 mm Hg by a noncontact tonometer. A slit-lamp examination showed an edematous left eyelid with conjunctival congestion and follicles (Figs. 1A, B). Scattered dendritic lesions with terminal bulbs and intraepithelial infiltrates were present throughout the cornea. However, the overall branching and terminal bulb formation were minimal (Figs. 1C, D). The immunochromatographic test (Checkmate Herpes Eye; Wakamoto Pharmaceutical Co, Tokyo, Japan) of the conjunctival smear showed positive results for HSV antigens. The eruption on the skin of the ear appeared in conjunction with conjunctivitis. Serum allergy testing was positive for house dust mite and cedar-specific IgE antibodies. The serum HSV IgG antibody was positive, but IgM was negative. Based on the abovementioned test results, the patient was diagnosed with HSV atypical dendritic keratitis with atopic diathesis.

As previously described, we evaluated the HSV DNA levels in the tears at the initial visit (day 7) and at days 11, 14, and 21 by real-time PCR (Fig. 1E). Topical acyclovir ointment, 5 times a day, was initiated on day 7. At this point, the HSV DNA expression in tears was $6.4 \times 10^2$ and $1.6 \times 10^5$ copies/sample in the right and left eyes, respectively. Oral valacyclovir was also prescribed at a dose of 1000 mg/d for 5 days owing to bilateral HSV DNA expression and the suspected herpes simplex eruption in her right ear. On day 11, the epithelial defect healed leaving behind subepithelial opacities. On day 14, multiple punctate subepithelial opacities (Figs. 2A, B) were seen, and the tear levels of HSV DNA expression were undetectable (Fig. 1E) in both eyes. A diagnosis of herpetic stromal keratitis onset was made, and the frequency of topical acyclovir ointment was reduced to thrice a day; 0.1% fluorometholone eye drops were added twice daily to prevent worsening of the corneal opacity. Multiple punctate subepithelial opacities persisted on day 21 (Figs. 2C, D). We reduced the frequency of topical acyclovir ointment from thrice to twice daily and continued 0.1% fluorometholone eye drops twice daily, after which the patient discontinued follow-up owing to unknown reasons.

**DISCUSSION**

We report a case of HSK in a patient who presented with atypical dendritic keratitis, and whose case evolved into stromal keratitis with multiple punctate subepithelial opacities. The atypical presentation is attributed to the sparse branching pattern and poor development of terminal bulbs in the dendrites. Patient-related factors that could have influenced the atypical presentation are atopic diathesis and the relative immune-deficient state consequent to systemic or topical corticosteroids or immunosuppressive agents. In some patients with HSK and atopic diathesis, bilateral atypical dendritic keratitis or stellate-like keratitis is associated with follicular conjunctivitis. In our patient, follicular conjunctivitis and dendritic keratitis occurred synchronously, with bilateral tear positivity for HSV DNA and positive antigen-specific IgE antibody titers in the serum. Atypical dendritic keratitis is also complicated by concurrent corneal disorders. However, our patient was a relatively healthy subject who wore contact lenses for refractive correction and had no underlying corneal disorders. Therefore, our final diagnosis was that of HSK with atypical dendritic keratitis that developed in the background of atopic diathesis.

Primary HSV-1 infection usually develops in childhood and remains unrecognized with no obvious clinical disease or
presents clinically as gingivostomatitis, infections of the upper respiratory tract, skin, or eye, including conjunctivitis and keratitis. Primary HSV-1 ocular infection may present as follicular conjunctivitis or various types of corneal epithelial keratitis including punctate and dendritic keratitis with concomitant vesicles or erosions of the eyelid margin. In patients with primary HSV-1 infection, subepithelial corneal opacities similar to that of adenovirus or chlamydia infections appear in approximately 20% of cases during the course of progression. However, this corneal finding is not typically observed in HSK owing to reactivated HSV. Okubo et al reported a suspected case of primary bilateral HSK with epithelial defects simulating the arabesque pattern, which healed with topical steroids and developed into multiple punctate subepithelial opacities. Atypical dendritic lesions with poor terminal bulb formation and intraepithelial infiltration are seen in the arabesque pattern. In our case, HSV antibody titers (HSV IgM negative and HSV IgG positive) indicated a reactivation of latent HSV. The reactivation of HSV in patients with atopic diathesis may lead to similar clinical findings as primary infection.

Real-time PCR measures HSV DNA levels in tears, and immunochromatographic methods detect HSV antigens in corneal specimens. These tools are useful in clinical practice for diagnosing HSK. We have previously reported the tear levels of HSV DNA in typical dendritic keratitis to be $10^2$–$10^7$ (median: $2.3 \times 10^5$) copies/sample by real-time PCR. We reported that the median HSV DNA values in the unaffected eyes in the dendritic keratitis group were $2.2 \times 10^3$ copies/sample, and the positivity rate was 27.8%. In our case of atypical dendritic keratitis, the HSV DNA levels in tears of affected eye were $10^4$ copies/sample. In addition, immunochromatography was positive and aided in our diagnosis. The tear level of HSV DNA in the unaffected eye was $10^2$ copies/sample, and this can be explained by spontaneous ocular shedding of the virus.

During the phase of multiple punctate subepithelial opacities in the affected eye, the tears had undetectable levels of HSV DNA. The lower detection limit value was set at 10 copies/sample in our real-time PCR. The HSV DNA in tears of disciform keratitis ranges between undetectable levels to $9.2 \times 10^2$ copies/sample. Therefore, as the ocular surface viral load tends to decrease from the stage of atypical dendritic keratitis to that of multiple punctate subepithelial opacities, it may be presumed that the latter develops in an environment with a lower HSV burden. We also speculate that similar to disciform keratitis, multiple punctate corneal subepithelial opacities could result from an immune response to HSV.

To conclude, we have successfully detected the shift from atypical dendritic keratitis to multiple punctate corneal subepithelial opacities in HSK by quantitative measurement of HSV DNA in tears using real-time PCR. The HSV DNA levels in tears need to be estimated promptly to facilitate the accurate diagnosis of corneal epithelial keratitis and to identify the optimal timings for cessation of antiviral therapy and steroid use in epithelial and stromal keratitis, respectively.

REFERENCES