Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein in the Ocular Tissues of a Patient Previously Infected With Coronavirus Disease 2019

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IMPORTANCE Coronavirus disease 2019 (COVID-19) has been recognized as a pandemic by the World Health Organization. Whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can also infect tissues besides the respiratory system, such as the ocular tissues, remains unclear.

OBJECTIVE To determine whether SARS-CoV-2 exists intracellularly in the ocular tissues of a patient previously infected with COVID-19.

DESIGN, SETTING, AND PARTICIPANTS This case study analyzed a patient previously infected with COVID-19 who had an acute glaucoma attack during her rehabilitation. Plasma samples and tissue specimens, including ones from the conjunctiva, anterior lens capsule, trabecular meshwork, and iris, were collected during phacoemulsification and trabeculectomy surgery. Specimens from another patient who had glaucoma but not COVID-19 were used as a negative control.

MAIN OUTCOMES AND MEASURES Specimens were analyzed using hematoxylin-eosin staining. The nucleocapsid protein antigen of SARS-CoV-2 was measured in the conjunctiva, trabecular meshwork, and iris using immunofluorescence and immunohistochemistry. The expression of angiotensin-converting enzyme 2 receptor on conjunctiva was measured using immunohistochemistry.

RESULTS The patient with a previous COVID-19 infection was female and 64 years old, and the control patient without a COVID-19 infection history was male and 61 years old. The nucleocapsid protein antigen of SARS-CoV-2 was detected on the cells of the conjunctiva, trabecular, and iris of the patient infected with COVID-19 but not in the control participant, while angiotensin-converting enzyme 2 receptor proteins were detected on the conjunctiva of both patients.

CONCLUSIONS AND RELEVANCE The nucleocapsid protein antigen of SARS-CoV-2 existed intracellularly in the ocular tissues of a patient previously infected with COVID-19. Thus, SARS-CoV-2 can also infect ocular tissues in addition to the respiratory system.
Methods

Ethical approval was obtained from the Ethics Committee of the General Hospital of the Central Theatre. Written informed consent was given by both patients. A 64-year-old woman presented with dry cough for 5 days and diarrhea for 9 days before she was admitted to the hospital on January 31, 2020. On the day of admission, her temperature was 37.8 °C and her oxygen saturation was 98%. Laboratory tests showed that her C-reactive protein concentration was elevated to 1.25 mg/dL (normal range, 0-1.0 mg/dL [to convert to milligrams per liter, multiply by 10]). A complete blood cell count showed a leukocyte count of 7270 cells/μL (normal range, 400-10 000 cells/μL [to convert to cells × 10⁹ per liter, multiply by 0.001]). Computed tomography scans of her chest showed ground-glass opacity in her lower bilateral lobes. Based on RT-PCR analysis of throat swab samples (eMethods 1 in the Supplement), she was diagnosed with COVID-19. She was then admitted to the hospital to receive antiviral and antibacterial treatment, as well as supplemental oxygen, as empirical therapy. During her hospitalization, the patient had no serious respiratory or ocular symptoms. By day 18, her symptoms were fully resolved, and the throat swab RT-PCR tests, which were performed on February 18 and 20, indicated negative results.

On February 28, she began experiencing persistent left eye pain and visual acuity loss. Three days later, the same symptoms developed in her right eye. She was admitted to the ophthalmology clinic on March 8. At presentation, her visual acuity was light perception bilaterally. The intraocular pressure was 50 mm Hg in both eyes. A slitlamp examination showed a flat anterior chamber, and the intraocular pressure (IOP) was 50 mm Hg in both eyes. A complete blood cell count showed a leukocyte count of 7270 cells/μL (normal range, 400-10 000 cells/μL [to convert to cells × 10⁹ per liter, multiply by 0.001]). Computed tomography scans of her chest showed ground-glass opacity in her lower bilateral lobes. Based on RT-PCR analysis of throat swab samples (eMethods 1 in the Supplement), she was diagnosed with COVID-19. She was then admitted to the hospital to receive antiviral and antibacterial treatment, as well as supplemental oxygen, as empirical therapy. During her hospitalization, the patient had no serious respiratory or ocular symptoms. By day 18, her symptoms were fully resolved, and the throat swab RT-PCR tests, which were performed on February 18 and 20, indicated negative results.

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Discussion

The patient under study was in the recovery period of infection and thus the 3-time nucleic acid testing of her swabs had been negative. Fasting blood samples of approximately 5 mL were drawn from both participants to detect SARS-CoV-2 IgG and IgM antibodies (Innovita Biological Technology Co Ltd) and nucleocapsid protein (NP) antigen (Bioeasy Biotechnology Co Ltd). All specimens were placed in a freezing box at 4 °C and transported to the laboratory within 30 minutes. The specimens were embedded in paraffin, cut into 4-μm slices, and mounted onto polylysine-coated glass slides stained with hematoxylin-eosin. The NP antigen of SARS-CoV-2 was detected on the conjunctiva, trabecular, and iris specimens using immunohistochemistry or immunofluorescence, while angiotensin-converting enzyme 2 (ACE2) receptor protein on the conjunctiva was detected using immunohistochemistry. Additional details are available in eMethods 2 in the Supplement.

Results

For both patients, the NP antigen of SARS-CoV-2 was not detected in the plasma. Additionally, IgG antibody tests had a positive result and IgM antibody tests had a negative result in the patient with COVID-19, while IgG and IgM antibody tests in the control participant both had negative results. Since no cell structure was found in the anterior capsule of the lens under hematoxylin-eosin staining, neither immunohistochemical nor immunofluorescence measurements were performed. Immunohistochemistry was performed for conjunctiva specimens from both patients; the results are shown in Figure 1. The NP antigen was present intracellularly in the conjunctiva collected from the patient previously infected with COVID-19 but absent in the conjunctiva tissue collected from the control patient. The ACE2 receptor proteins could be detected in the conjunctiva cells from both patients.

Immunofluorescence analysis was performed for the iris and trabecular from both patients; the results are shown in Figure 2. Iris and trabecular meshwork samples from the patient previously infected with COVID-19 showed positive staining for the NP antigen, while the iris and trabecular meshwork samples from the control participant did not show NP antigen staining.

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Key Points

Question Does severe acute respiratory syndrome coronavirus disease 2 (SARS-CoV-2) exist in the ocular tissues of a patient with coronavirus disease 2019 (COVID-19)?

Findings In this case study, nucleocapsid protein antigens were detected on the cells of the conjunctiva, iris, and trabecular meshwork of a patient with a COVID-19 infection, and these antigens were absent on the specimens from the control patient. In addition, angiotensin-converting enzyme 2 receptor proteins were detected in the conjunctiva cells of this patient and a control participant.

Meaning Nucleocapsid protein antigens of SARS-CoV-2 existed in the inner ocular tissues of a patient previously infected with COVID-19, which implied that SARS-CoV-2 can infect ocular tissues as well as the respiratory system.
negative results. The method used to detect the SARS-CoV-2 NP antigen in this study was similar to the method used in a previous investigation of lung and kidney autopsy specimens. The specimens from the patient without an infection in this study showed no SARS-CoV-2 NP antigen expression; the evidence provided was relatively reliable.

In the patient previously infected with COVID-19, the SARS-CoV-2 NP antigen was found in the conjunctival, trabecular, and iris tissues. This indicated that SARS-CoV-2 may exist intracellularly in the inner ocular tissues as well as on the ocular surface. Based on these results, the eye is also one of the target organs for the viral infection in addition to the lungs. Although the method by which the virus enters the eye is still unclear, it could theoretically enter the inner eye tissues in 2 ways. First, SARS-CoV-2 could enter the inner eye tissue via the ACE2 receptor on the surface of the conjunctiva. This study's results showed that ACE2 receptor proteins are expressed on the conjunctival cell, seemingly supporting this hypothesis. However, this hypothesis can only be confirmed through animal experiments—for example, ones that detect the virus in the intraocular tissue after conjunctiva inoculation.

Second, the virus may spread systemically to end organs as a result of the primary respiratory infection. However, when the patient presented at the ophthalmology clinic, the possible viremia was already in the recovery stage, and the SARS-CoV-2 NP antigen test and IgM antibody in the plasma showed negative results. These findings do not seem to support this second transmission-route hypothesis.

Limitations
The patient under study was not in the acute period of infection. The transmission route was short of definite evidence.

Conclusions
The viral antigen detected in the eye of the patient 2 months after infection should prompt future investigations. These investigations should aim to determine whether the viral NP antigens that remain in the eye over time cause damage to the ocular structure or function, represent the presence of active viral residues in organs (not only the eyes), and are still infectious.


