American Journal of Ophthalmology Case Reports 22 (2021) 101074

Contents lists available at ScienceDirect

American Journal of Ophthalmology Case Reports

journal homepage: www.ajocasereports.com/

Detection of SARS-CoV-2 RNA in the corneal epithelium of a patient after recovery from COVID-19

Irene C. Kuo [a,*], Heba H. Mostafa [b]

[a] Wilmer Eye Institute, Department of Ophthalmology, Johns Hopkins University School of Medicine, USA
[b] Division of Medical Microbiology, Department of Pathology, Johns Hopkins University School of Medicine, USA

ARTICLE INFO

Keywords:
COVID-19
Uveitis
Keratouveitis
Cornea
SARS-CoV-2
Coronavirus

ABSTRACT

Purpose: To report a case of a patient presenting with unilateral keratouveitis associated with ocular hypertension six weeks after being discharged from the hospital for COVID-19. Ocular specimens were obtained for testing.
Observations: A 69-year-old African American woman developed poor vision while hospitalized for COVID-19 in April but did not seek ophthalmic care until end of May. She had an edematous cornea, stromal keratitis, and highly elevated intraocular pressure by June. After lack of response to oral valacyclovir, aqueous fluid and swabs of her conjunctiva and limbal epithelium with corneal epithelium anterior to the limbus were sent for real-time polymerase chain reaction (PCR) for herpes simplex virus, herpes zoster virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Epithelium from the cornea and limbus was positive for SARS-CoV-2 by PCR; specimens from the other two ocular sites were negative. All specimens were negative for herpes simplex virus and varicella zoster virus. The patient refused further treatment despite intraocular pressure above 50 mm Hg at last follow-up.
Conclusions and Importance: Although SARS-CoV-2 and severe acute respiratory syndrome coronavirus (SARS-CoV) have been detected by PCR in the conjunctiva and tears of patients with acute respiratory infection, presence in corneal tissue has not been described. In addition, no one has studied whether ocular tissues in convalesced patients can harbor viral RNA. Here we describe unilateral keratouveitis in a convalesced patient whose corneal epithelium/limbal tissue was positive for SARS-CoV-2 by PCR. Further investigation is required to determine whether active viral replication or viral remnants account for this result.

1. Introduction

Although SARS-CoV and SARS-CoV-2, the coronaviruses causing Severe Acute Respiratory Syndrome (SARS)[1] and coronavirus disease 2019 (COVID-19), respectively,[2,3] have been detected by polymerase chain reaction (PCR) in tears of infected patients, little is known about possible ocular reservoirs in convalesced patients. In this case report, diagnostic sampling was performed as part of clinical care. The Institutional Review Boards of Johns Hopkins Medicine ruled that approval was not required.

1.1. Case report

In April 2020, a previously healthy 69-year-old African American woman presented to a hospital with shortness of breath and was diagnosed with COVID-19 based on nasopharyngeal swabbing and radiographic findings. During her two-week hospitalization, she experienced sudden left eye visual decline accompanied by redness, sharp pains, and light sensitivity. She did not complain about her vision until discharge at which time an outpatient ophthalmology appointment was made. However, she was forced to self-quarantine for two weeks, then postponed her appointment.

In late May, an ophthalmologist examined her. Visual acuity was 20/30 in the right eye and hand motions in the left eye. Schiotz indentation tonometry was 14 mm Hg in each eye. Left eye findings were significant for anterior chamber cell, corneal edema, and cataract with no view of the posterior segment. She was started on topical prednisolone acetate 1% every 2 hours but did not improve after 4 days and was told to seek a second opinion.

In mid-June, an optometrist at the Wilmer Eye Institute saw the patient. She had mild left eye discomfort. Visual acuities were unchanged; rebound tonometry (iCare, Finland) was 14 and 44 mg Hg,

* Corresponding author. Wilmer Eye Institute, 4924 Campbell Blvd. #100, Baltimore, MD, 21236, USA.
E-mail address: ickuo@jhmi.edu (I.C. Kuo).

https://doi.org/10.1016/j.ajoc.2021.101074
Received 30 October 2020; Received in revised form 7 March 2021; Accepted 10 March 2021
Available online 16 March 2021

right and left eyes, respectively. Topical steroid was decreased to 3 times a day; brimonidine 0.2% twice a day was started.

Visual acuities were unchanged a day later with the ophthalmologist, but the left eye was more comfortable. She denied a history of herpes zoster. The left pupil displayed an afferent pupillary defect. Tonometry was 16 and 22 mm Hg, right and left eyes. The left eye displayed 2+ bulbar conjunctival injection, 2+ anterior chamber cell, and marked corneal edema with decreased corneal sensation and microcystic epithelial involvement that prevented posterior segment visualization (Fig. 1). The edema was associated with diffuse stromal keratitis. Corneal sensation was decreased. Both chambers were deep. Prednisolone acetate 1% was increased to 6 times a day. Oral valacyclovir 1000 mg three times a day was initiated for possible herpes simplex or zoster keratouveitis.

A week later, the left eye remained comfortable but showed no other improvement. Intraocular pressure had risen to 44 mm Hg. The decision was made to obtain ocular tissue sampling for analysis. A flocked swab included with the viral transport medium was used to collect a superficial specimen of the inferior forniceal conjunctiva as well as of the limbal epithelium and corneal epithelium anterior to the limbus. These ocular specimens and aqueous fluid were submitted for PCR testing. For the swab of the corneal/limbal epithelium, an epithelial defect was created in edematous cornea. The Centers for Disease Control and Prevention (CDC) SARS-CoV-2 real-time PCR panel assay was used.[4,5] This assay's oligonucleotide primers and probes target 2 regions of the nucleocapsid (N) gene as well as detect the human RNase P gene (RP) for extraction and specimen quality evaluations.[4,5] Therefore, the assay was performed in three separate reactions per specimen for each target (N1, N2, and the internal control RP).[5] The swab of epithelium from the limbus and cornea was positive for SARS-CoV-2 in the N1 and N2 target regions of the nucleocapsid gene and the human RNase P gene (cycle threshold values of 32.11, 31.69, and 28.45, respectively). The conjunctival swab and aqueous fluid were negative. All samples were negative for herpes simplex virus-type 1 or herpes zoster virus.

The patient was sent to the Glaucoma service where tonometry was 18 and 42 mm Hg. Gonioscopy of the right eye revealed a closed angle; laser iridotomy was performed. Intraocular pressure decreased to 20 mm Hg in the left eye on 3 medications but rose to 51 mm 10 days later. The patient refused any further treatment.

2. Discussion

To the best of our knowledge, this is the first report of SARS-CoV-2 RNA detection in the cornea. It also may be the first to show that the corneal epithelium/limbus can be a reservoir of viral RNA in a patient who has recovered from COVID-19. SARS-CoV and SARS-CoV-2 have been found in tears of patients with active pulmonary disease and conjunctivitis[2,3] but have not been studied in eyes of convalesced patients. Although the patient's presentation raised suspicion for keratouveitic glaucoma, to date coronavirus-associated uveitis has been described only in cats,[6] ocular manifestations of which include conjunctivitis, pyogranulomatous anterior uveitis, choroiditis with

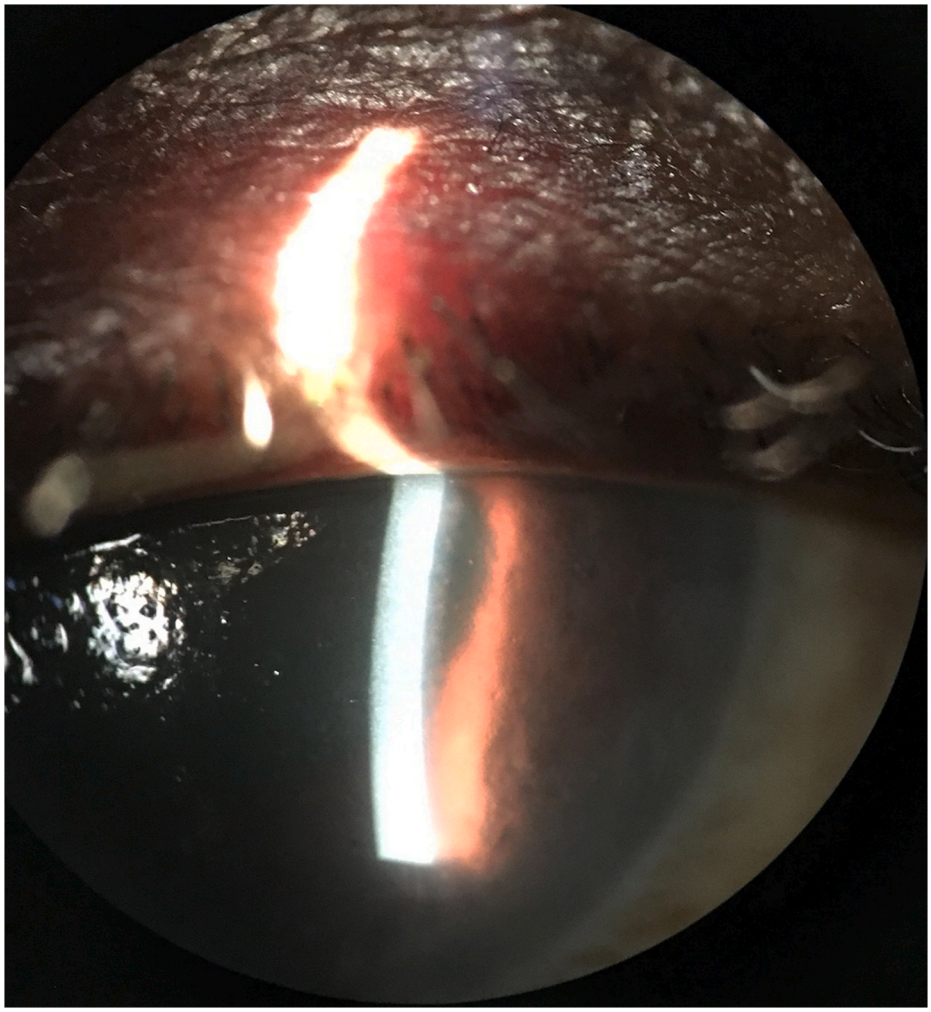

Fig. 1. Biomicroscopic slit-lamp photograph of left eye showing diffuse corneal edema and keratic precipitates, anterior chamber inflammation, and cataract.

retinal detachment, and retinal vasculitis.[6] The patient did not respond to oral valacyclovir prescribed for presumed herpes simplex or varicella zoster keratouveitis.

For the CDC PCR assay, the primer-probe mixes for N1 and N2 specifically detect SARS-CoV-2; the primer-probe mix for the human RNase P gene (RP) serves as an internal control by detecting human nucleic acid; and the fourth reagent is noninfectious positive control material.[7] The cycle threshold is usually used as a qualitative measure of the target region's concentration; lower values represent qualitatively higher viral RNA loads. A value less than 40 is clinically reported as PCR positive. All values for the N1 and N2 regions of the nucleocapsid and for RP from this patient's corneal epithelium/limbal swab were positive for SARS-CoV-2.

It is unclear whether this patient's presentation and RT-PCR positivity represent viral replication (and therefore infectious virus) or a post-COVID-19 inflammatory reaction due to remnant RNA. Presently tissue banks are not testing tissue for SARS-CoV-2. A recent small study did not detect SARS-CoV-2-RNA in post-mortem cornea, conjunctiva or aqueous humor of decedents of COVID-19.[8] Even had the flocked swab penetrated this patient's Bowman's membrane, (difficult to ascertain given the amount of corneal edema), the specimen was mostly epithelial. Epithelial positivity may not be an issue of concern for some in terms of tissue banking, but donor epithelium can survive corneal transplantation until it is eventually replaced by host epithelium.

Both ACE2 (the receptor for SARS-CoV-2) and TMPRSS2 (a cell surface-associated protease that facilitates viral entry after the viral spike protein binds to ACE2) are expressed in human corneal epithelium, endothelium, and limbus.[9] Investigators discovered that immunohistochemical staining for ACE2 was especially prominent in the superficial conjunctival and corneal epithelial surface of the limbus and largely absent from the corneal stroma.[9] The fact the conjunctival swab sample was negative for SARS-CoV-2 by PCR and the corneal epithelium specimen was positive in this patient argues against a nasopharyngeal contaminant. We did not obtain nasopharyngeal testing during her ophthalmologic visits; she had had no respiratory symptoms in the 2 months since hospital discharge for COVID-19. According to the CDC, "persons with more severe to critical illness or severe immunocompromise likely remain infectious no longer than 20 days after symptom onset,"[10] although recovered patients "can continue to shed detectable SARS-CoV-2 RNA in upper respiratory specimens for up to 3 months after illness onset" (albeit at much lower concentrations).[10–12] Therefore, nasopharyngeal swabbing when she presented to us might not have been very informative. Moreover, given the low proportion (1–5%) of PCR-positive conjunctival swabs from hospitalized COVID-19 patients,[13,14] this patient's negative conjunctival swab is not surprising. The result of the negative aqueous PCR has unclear implications as the diagnostic utility of PCR from intraocular fluids in uveitis has limitations.[15,16] Positivity may depend on timing of sampling with respect to onset of signs and symptoms, etiology of the uveitis, and nature of the inflammation (acute, subacute, or chronic).[15,16] Therefore, positive PCR results from intraocular fluids can confirm a final clinical diagnosis, but negative results cannot exclude infectious disease.[15]

The positive corneal epithelial/limbal epithelial result is curious as neither corneal involvement by SARS-CoV-2 nor recovery of viral RNA from the cornea has been reported. There was no justification to sample the fellow (unaffected) eye for PCR for SARS-CoV-2.

Her findings resembled herpes simplex stromal keratitis, which may occur as a progression from infectious epithelial keratitis, be the primary manifestation of keratitis, or more typically, be a recurrent condition and requires both antiviral and corticosteroid therapy. Based on CDC information, reinfection by SARS-CoV-2 appears unlikely in the first 3 months after initial infection. Therefore, perhaps this patient's keratouveitis represents a post-COVID-19 inflammatory reaction following primary infection of the ocular externa. Despite evidence of tropism of coronaviruses for portions of the central nervous system (namely, the olfactory nerve for both SARS-CoV and SARS-CoV-2),[17] SARS-CoV-2 seems more similar to other common respiratory viral pathogens (e.g., influenza) than to pathogens with specific neurotropism (e.g., herpes simplex).[18] The patient described onset of eye pain and redness 4 weeks before ophthalmic examination. Marked corneal edema, stromal keratitis, and anterior chamber reaction were present before tonometry became elevated, making primary angle closure less likely than keratouveitic glaucoma.

Detection of SARS-CoV-2 RNA in ocular tissue may spur interest in testing ocular specimens in cell culture lines and development of clinical assays. Without a clinical assay for ocular samples, respiratory assays must be used on a research-use only basis, as has been done in all testing to date of conjunctiva and tears of patients with COVID-19.[19]

Patient consent

As no identifying information is disclosed, patient consent was not obtained.

Funding

None.

Authorship

Both authors attest that they meet the current ICMJE criteria for Authorship.

Declaration of competing interest

None.

Acknowledgment

We thank Michael Forman of Johns Hopkins Medical Microbiology for performing the PCR analysis.

References

1. Loon SC, Teoh SC, Oon LL, et al. The severe acute respiratory syndrome coronavirus in tears. *Br J Ophthalmol*. 2004;88(7):861–863.
2. Zhou Y, Duan C, Zeng Y. Ocular findings and proportion with conjunctival SARS-COV-2 in COVID-19 patients. *Ophthalmology*. 2020;127:982–983.
3. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol*. 2020;92(6):589–594.
4. CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel: for emergency use only. February 4, 2020. Updated July 13, 2020. Available at: https://www.fda.gov/media/134922/download. Accessed August 31, 2020.
5. Uhteg K, Jarrett J, Richards M, et al. Comparing the analytical performance of three SARS-CoV-2 molecular diagnostic assays. *J Clin Virol*. 2020;127:104384.
6. Doherty MJ. Ocular manifestations of feline infectious peritonitis. *J Am Vet Med Assoc*. 1971;159:417–424.
7. CDC's diagnostic test for COVID-19 only and supplies. July 15, Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html; 2020. October 8. 2020.
8. Bayyoud T, Iftner A, Iftner T, et al. Absence of severe acute respiratory syndrome-coronavirus-2 RNA in human corneal tissues. *Cornea*. 2020. Jun 29. Online ahead of print.
9. Zhou L, Zhenhua Xu Z, Gianni M, et al. CE2 and TMPRSS2 are expressed on the human ocular surface, suggesting susceptibility to SARS-CoV-2 infection. *Ocul Surf*. 2020;18(4):537–544.
10. Duration of isolation and precautions for adults with COVID-19. Updated September 10, 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html. Accessed October 9, 2020.
11. Li N, Wang X, Lv T. Prolonged SARS-CoV-2 RNA shedding: not a rare phenomenon. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25952. Apr 29.
12. Korea centers for disease control and prevention. May 19 *Findings from Investigation and Analysis of re-positive cases*; 2020. Available at: https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1. Accessed October 10, 2020.
13. Zhou Y, Duan C, Zeng Y, Tong Y, et al. Ocular findings and proportion with conjunctival SARS-COV-2 in COVID-19 patients. *Ophthalmol Times*. 2020;127(7):982–983.

14. Wu P, Duan F, Luo C, et al. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in hubei province, China. *JAMA Ophthalmol*. 2020. https://doi.org/10.1001/jamaophthalmol.2020.1291. Mar 31.
15. Harper TW, Miller D, Schiffman JC, Davis JL. Polymerase chain reaction analysis of aqueous and vitreous specimens in the diagnosis of posterior segment infectious uveitis. *Am J Ophthalmol.* 2009;147(1):140–147.
16. Anwar Z, Galor A, Albini TA, Miller D, Perez V, Davis JL. The diagnostic utility of anterior chamber paracentesis with polymerase chain reaction in anterior uveitis. *Am J Ophthalmol.* 2013;155(5):781–786.
17. Hwang CS. Olfactory neuropathy in severe acute respiratory syndrome: report of a case. *Acta Neurol Taiwanica.* 2006;15(1):26–28.
18. Iadecola C, Anrather J, Kamel H. Effects of COVID-19 on the nervous system. Oct 1 *Cell.* 2020;183(1):16–27. e1.
19. Kuo IC. A Rashomon moment? ocular involvement and COVID-19. *Ophthalmology*. 2020;127(7):984–985.