



# Intraocular Fluid Sampling In Patients with Sight Threatening COVID-19 Ophthalmic Sequelae

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**Keywords:** Corona virus disease; COVID-19; Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2); Reverse Transcriptase Polymerase Chain Reaction (RT-PCR); Intraocular fluid analysis; Ocular sequelae.

**Abbreviations:** (COVID)-19: Corona Virus Disease; SARS- CoV-2; Severe Acute Respiratory Syndrome Corona Virus; SARS- CoV-2 RBD: Severe Acute Respiratory Syndrome Corona Virus Receptor Binding Domain; RT- PCR: Reverse Transcriptase Polymerase Chain Reaction; ACE: Angiotensin Converting-Enzyme; TM-PRSS2: Trans Membrane Serine Protease 2; RNA: Ribo Nucleic Acid.

## Abstract

**Background:** Coronavirus disease 19 (COVID-19) has been associated ophthalmic sequelae following recovery.

**Purpose:** This study explores the possible role of the severe acute respiratory syndrome corona virus (SARS-CoV-2) in ophthalmic manifestations by analyzing the ocular samples.

**Methods:** Retrospective case series

**Results:** Five Asian male patients, previously diagnosed with COVID-19 with poor visual acuity and history of previous COVID-19 were included in this study. The onset of ocular symptoms after the diagnosis of COVID-19 ranged between 2-50 days (median 7 days).

The time from COVID-19 diagnosis to ocular sampling was 14-60 days (median 27 days) and from ocular symptoms to ocular sampling was 1-50 days (median 4 days). The age ranged between 37-66 years, with two patients with pre-existing diabetes, and two developing diabetes after having been treated with steroids during COVID-19 management. While all the patients underwent anterior chamber paracentesis, in three of them vitreous sampling was also done.

**Conclusions:** Though our patients had prior use of topical medication and we had no positive controls, this small study of a diverse group of sight threatening manifestations was not able to demonstrate severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) in intraocular fluid samples.



## Introduction

Post Coronavirus disease 2019 (COVID-19) ocular sequelae reported in medical literature include conjunctivitis, new or re-activation of quiescent anterior uveitis, vitritis, panuveitis, cotton wool spots, retinal hemorrhages, retinal artery and vein occlusion, ophthalmic artery occlusion, papillophlebitis, multifocal chorioretinitis, central serous chorioretinopathy and Adie's syndrome [1-7]. Ocular manifestations can precede COVID-19 as reported in a single case report although it cannot be conclusively proven [8]. These manifestations can either be due to the virus itself or secondary to the hypercoagulable or inflammatory state induced by COVID-19 [1]. A confirmatory test of the underlying pathogenesis could be the presence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in intraocular fluids, given the isolation of the virus from the conjunctiva and presence of SARS-CoV-2 from the vitreous [9,10]. The absence of the virus in intraocular fluids may be inconclusive if the virus is restricted to the retinal vasculature by the blood-retinal barrier.

## Materials and Methods

Inclusion criteria were confirmed patients of COVID-19 who presented to our center with sight threatening manifestations, a corrected distance visual activity (CDVA) <20/200 and those willing to undergo intraocular fluid sampling for diagnostic purposes. Anterior chamber samples were obtained in suspected endogenous endophthalmitis/panophthalmitis to seek an infective cause. In a patient with central retinal artery occlusion, anterior chamber paracentesis was used as a treatment modality. In a patient with hyphema and vitreous hemorrhage, the sample was collected during anterior chamber wash. In two patients (patient 4 and 5) vitreous sample was taken during pars plana vitrectomy. For one patient (patient 2) RT-PCR was done for the vitreous sample and eviscerated tissue. Samples were collected in a 1ml tuberculin syringe, capped, and transported in a viral transport medium (Bhat Biotech™) to the laboratory maintaining a temperature between 2-8° centigrade for RT-PCR (reverse transcriptase- polymerase chain reaction) to detect SARS-CoV-2 [11,12]. TaqPath™ COVID-19 Multiplex Diagnostic Solution from Thermo Fisher Scientific™ was used in our series for multiplex real-time RT-PCR testing to allow qualitative detection of nucleic acid from SARS-CoV-2 [12]. The primer and probe sets used with the test were designed to amplify and detect three regions of the SARS-CoV-2 single stranded RNA genome: the Orf1ab, N gene and S gene [12].

## Results

Five Asian male patients presented to our tertiary eye center with ocular symptoms like severe pain, acute loss of vision, redness and watering. They had been previously diagnosed and treated for COVID-19 elsewhere. The time to onset of ocular symptoms after diagnosis of COVID-19 ranged between 2-50 days (median 7 days). Patient 5 presented to our center for a second opinion after a delay as he was seeking treatment elsewhere, The time from COVID-19 diagnosis to ocular sampling was 14- 60 days (median 27 days) and the time between ocular symptoms to ocular sampling was 1- 50 days (median 4 days). The age of patients ranged between 37-66 years. Two patients had pre-existing diabetes and two developed diabetes after treatment with steroids during COVID-19 management. At the time of presentation of ocular symptoms, in all patients the SARS-CoV-2 receptor binding domain (SARS- CoV-2 RBD) total (IgG and IgM) antibodies was high (>10). Clinically, each of the

patients were diagnosed as follows:

Case 1- bilateral panuveitis with optic nerve edema and unilateral central retinal artery occlusion [1].

Case 2- bilateral panophthalmitis

Case 3- Unilateral hyphema with secondary glaucoma and bilateral retinal vasculitis with vitreous hemorrhage following anticoagulant treatment for hypercoagulability for COVID-19

Case 4- unilateral endogenous bacterial endophthalmitis [6].

Case 5- unilateral fungal endogenous endophthalmitis

All the patients ocular samples were negative for RT-PCR for SARS-CoV-2. Nested PCR for Cases 2 and 4 were positive for eubacterial genome and Cases 2 and 5 for panfungal genome.

**Table 1** shows the details of ocular sampling.

**Table 1:** Shows three patients with infective aetiology who underwent ocular sampling.

Patient	Diagnosis	Ocular sampling
Case 2	Panophthalmitis OU	Vitreous Tap OD <b>Gram positive cocci and Gram negative bacilli</b> <b>Klebsiella pneumonia</b> <b>PCR positive for panfungal genome</b> AC Tap OU <b>Gram positive cocci in singles and pairs</b>
Case 4	Endophthalmitis OD	AC tap- gram positive cocci Vitreous tap- Gram positive cocci, no growth, <b>PCR for Eubacteria POSITIVE</b>
Case 5	Endophthalmitis OS	AC tap- <b>PCR for panfungal genome - Positive</b>

2 other patients who underwent AC chamber tap/wash were not subjected to infective screening.

All the five patient's ocular samples were subjected to RT-PCR for SARS-CoV-2, which were negative.

OD: Right eye; OS: Left eye; OU: Both eyes; AC Tap: Anterior chamber tap; PCR: Polymerase Chain Reaction; RT- PCR: Reverse transcriptase Polymerase Chain Reaction; SARS-CoV-2: Severe acute respiratory syndrome Corona virus 2.

## Discussion

Transmission of SARS-CoV-2 is thought to spread from person to person through respiratory droplets or close contact [13]. Napioli et al explored the spread of SARS-CoV-2 through the ocular route and believe that there is a low risk of coronavirus spread through tears. Nonetheless, they advocate eye protection with goggles or a face shield to prevent contamination of the ocular surface from external droplets or aerosols [14,15]. They also suggested conjunctival and nasopharyngeal swabs screening for high-risk cases requiring pre- and post-surgical drug prophylaxis for intraocular surgeries. SARS-CoV-2 binds to the host cell via the angiotensin converting-enzyme (ACE2) receptor and the transmembrane serine protease (TMPRSS2), which is a protease receptor that enables the infection of human cells [15,16]. These receptors are expressed with varying degrees in different tissues [17]. Conjunctival epithelial cells and retinal pigment epithelium and choroid are known to express ACE2 receptor in humans [18,19].

Corneal limbal stem cells contain ACE2 receptors and TMPRSS2 protein, which can facilitate breaching of the ocular surface by the SARS-CoV-2 and potentiate the spread of the virus

to other parts of the body through the blood stream and/or the nervous system (ophthalmic branch of trigeminal nerve) [20,21]. There is no current evidence that in humans SARS-CoV-2 can enter the eye or spread to the brain through corneal nerves. However, beta coronaviruses related conjunctivitis, uveitis, retinitis and optic neuritis have been reported in some animal models (feline and murine), thus suggesting that in some mammals these viruses can penetrate the eye [22]. Few studies have demonstrated viral RNA in the conjunctiva, aqueous and vitreous [10, 23-25]. Koo et al detected SARS-CoV-2 RNA in the aqueous despite a negative nasal swab testing suggesting that the virus may persist in immune-privileged spaces despite an absence of symptoms [23]. In another patient with bilateral panuveitis and neuro-retinitis, the RT-PCR for SARS-CoV-2 was positive in the vitreous sample [24]. In one study, only one in 45 patients (2.23%) had detectable levels of SARS-CoV-2 in conjunctival swabs using RT-PCR in COVID-19 positive patients [25]. Of the three post COVID-19 pneumonia patients with endogenous endophthalmitis, one patient's vitreous demonstrated the presence of SARS CoV-2 by RT-PCR [9]. List et al., [26]. In their study on SARS-CoV-2 infected patients with positive nasopharyngeal swab, none of the 16 aqueous and 16 vitreous samples subjected to RT-PCR were positive for SARS-CoV-2.

In a human ocular tissue post mortem study, a positivity rate for SARS-CoV-2 RNA of ~13% was noted. Of the ten COVID-19 who had donated their eyes, six had RT-PCR positive post-mortem nasopharyngeal swabs and eight exhibited high anti-SARS-CoV-2 IgG levels with three conjunctival, one anterior corneal, five posterior corneal, and three vitreous swabs testing positive for SARS-CoV-2 RNA [27]. In the epithelium of corneas that were procured without povidone iodine disinfection, SARS-CoV-2 spike and envelope proteins were detected [27]. In a retrospective analysis of nasopharyngeal swabs of all corneal donors who had expired from non-COVID causes, eleven (18.64%) were positive for SARS-CoV-2 [28]. It is possible that these donors could have had asymptomatic or undiagnosed coronavirus infection and hence recommended adding screening of nasopharyngeal swabs of all donors in the eye banking protocol. Interestingly they did not isolate any positive samples from the donor eyes. Casagrande et al [29,30]. In two different studies they were able to isolate SARS-CoV-2 RNA in 3 of 14 retinæ of deceased patients, in seven of 14 retinal biopsies and 10 of 13 optic nerve biopsies.

Factors that can influence the results of COVID-19 testing include the method of detection, preservation of the tissue, tested tissue samples may exhibit different expression of ACE2 receptors and TMPRSS2 with post mortem loss of receptor expression [17]. Reliability of tear samples in diagnosing SARS-CoV-2 may be questionable due to the inherent issues with the diagnostic kits and the viral load in the ocular samples [31]. Low level of viral detection can be due to various factors like the time required for maximum replication of the virus, the timing of sampling, time of presentation of the patient to the hospital and minimal secretion of the virus through conjunctival secretion [26].

Prior use of artificial tears with chloroquine, a known immunomodulator, antibiotics like azithromycin and tetracycline or eye drops which have preservatives like benzalkonium chloride; or antiseptics like povidone iodine and chlorhexidine, all with a potential antiviral effect can influence the detection of SARS-CoV-2 [32].

Primer and probe target mutations of SARS-CoV-2 genome

may lead to false-negative results [32]. The reasons could be related to the sample which may have amplification inhibitors, insufficient organisms (inappropriate collection), transportation, or handling issues [32]. This can be remedied by using multiple target gene amplification which would avoid invalid results. Early transport of the samples obtained by dacron or polyester flocced swabs will avoid false negative results [32].

Besides having been treated with remdesivir for COVID-19, 4 patients in our series had been treated with topical moxifloxacin (preserved with benzalkonium chloride) and prednisolone acetate 1% eye drops, which could have influenced our results. However, it needs to be proven if the preservatives could have entered the aqueous or vitreous, thereby potentiating any antiviral effect. Our smaller series did not have a positive control which would rule out the presence of PCR inhibitors in the aqueous or vitreous.

### Conclusions

In our series of five patients, none of the intraocular samples were positive for SARS-CoV-2 RT-PCR. Three patients had intraocular inflammation and infection but unlikely to be directly due to SARS-CoV-2. Standardization of ocular specimen processing is needed to conclusively isolate SARS-CoV-2 in ocular specimens.

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