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Conjunctival carriage of SARS-CoV-2 using serial sampling: risk factors and protective factors

To date, there remain many unknowns regarding ocular involvement in COVID-19. Various studies have reported conflicting rates of conjunctival carriage of SARS-CoV-2 (Table 1). A cross-sectional study on 1099 patients in China found only 0.8% of COVID-19 patients developed conjunctival congestion,[1] whereas a recent meta-analysis found the pooled prevalence of ocular manifestations among COVID-19 patients was 5.5% and the sensitivity of detecting SARS-CoV-2 in ocular fluids was merely 0.6%.[2] The low detection rate may suggest a low incidence of viral infiltration into ocular surface or may be due to variations in sampling technique, sampling time window, and underrepresentation from critical cases. To provide an answer, we set out to standardize the sampling technique and sampling time to minimize the chance of false negative results and to gauge whether SARS-CoV-2 can really infect the ocular surface.

Methods

We conducted a prospective study by adopting standardized technique for conjunctiva sampling via clear protocol and instructional video (see supplementary video). Adequate tissue sampling was ensured by sweeping lower tarsal conjunctiva twice. In cases of unilateral eye redness, only the involved eye was sampled to avoid transmitting any microorganisms to the uninvolved eye.

Two serial eye swabs were taken, one on admission (presentation sample) and another before hospital discharge (convalescent sample), to gauge whether viral shedding was present on the conjunctiva at the beginning and end of the COVID-19 disease course. A total of 78 eye swab samples were obtained from 39 consecutive COVID-19 patients admitted to a regional hospital in Hong Kong. Patient's medical history, ocular symptoms, nasopharyngeal SARS-CoV-2 results, and personal hygiene habits were prospectively collected and analyzed. The eye swab specimens underwent real-time reverse transcription–polymerase chain reaction (rRT-PCR) testing using the *E* gene assay, where a 76-bp-long fragment from a conserved region in the *E* gene would be detected by labeled hydrolysis probes using real-time PCR. The reagent used was TIB MOLBIOL LightMix®-Roche Diagnostics, Modular SARS and Wuhan CoV E-gene, Cat.-No. 53-0776-96. The results of specimen for rRT-PCR of SARS-CoV-2 was reported with corresponding cycle thresholds for positive specimen.

Results

All 39 cases had proven COVID-19 disease by respiratory samples for SARS-CoV-2 RT-PCR (Table 2). The mean age

Table 1—Summary of existing studies on the presence of SARS-CoV-2 in ocular secretions

Reference	Sample Size	No. of patients with ocular symptoms	Methodology	Positive findings (RT-PCR for SARS-CoV-2)	Conclusion
Xia et al.[10]	30 patients (21 mild disease 9 severe disease)	1 (conjunctivitis)	Conjunctival swab	2 samples from 1 patient with conjunctivitis	SARS–CoV–2 may be detected in the tears and conjunctival secretions in NCP patients with conjunctivitis
Wu et al.[11]	38 patients	12 (conjunctivitis, chemosis)	Conjunctival swab	2	One-third of patients in the sample group had ocular symptoms; it is possible for the virus to be transmitted through the eyes
Zhou et al.[12]	121 (63 mild–moderate disease 58 severe disease)	8 (3 patients with redness, others included itchiness, tearing)	Conjunctival swab	1 patient with ocular symptoms, 2 patients without ocular symptoms	The proportion with positive results for SARS-CoV-2 RNA was significantly less in the conjunctival and nasopharyngeal specimens
Seah et al.[13]	17 patients (64 samples were obtained over the study period)	1 (conjunctivitis after admission)	Schirmer's strip (tears)	0	Transmission through tears regardless of the phase of infection likely is low
Zhou et al.[14]	63 confirmed cases	1 (conjunctivitis)	Conjunctival swab	1 definite positive, 2 probable positive PCR result (none had ocular symptoms)	2019–nCoV can be detected in the conjunctival sac of patients with NCP (novel virus pneumonia)
Hui et al.[15]	1	Ex vivo study	Researchers isolated SARS-CoV-2 from a patient with confirmed COVID-19 and assessed infection using ex vivo cultures of human conjunctiva		SARS-CoV-2 infected the conjunctival mucosa Conjunctival epithelium appear to be potential portals of infection for SARS-CoV-2
Li et al.[16]	49	0	Conjunctival swab	4 patients	SARS-CoV-2 can be present on the conjunctiva in the absence of ocular symptoms

RT-PCR, reverse transcription–polymerase chain reaction; NCP, novel coronavirus pneumonia; nCoV, novel coronavirus.

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Table 2—Demographic characteristics, ocular protection, and personal hygiene habits of 39 COVID-19 patients in our case series

	No. of patients (percentage of total)
Female	19 (48.71%)
Male	20 (51.28%)
Age	40 y (range: 19–72)
Medical comorbidity	
HT	4 (10.26%)
Multiple sclerosis	1 (2.63%)
Hx of thyrotoxicosis	2 (5.13%)
DM	1 (2.63%)
COVID disease severity	
Mild	29 (74.36%)
Moderate	6 (15.38%)
Severe	3 (7.69%)
Critical	1 (2.56%)
Ocular symptoms	
Epiphora	4 (10.26%)
Itchiness	3 (7.69%)
Foreign body sensation	2 (5.13%)
Redness	1 (2.56%)
Discharge	1 (2.56%)
Eye protection and personal hygiene habits (protective factors)	
Spectacles (total)	30 (76.92%)
Full-time glasses	16 (41.03%)
Reading glasses/PRN	14 (35.90%)
Googles	3 (7.69%)
Hand washing frequency	
Hourly	18 (46.15%)
4–5 times/day	21 (53.85%)
Mobile phone cleaning with alcohol pad/spray	30 (76.92%)
Risk factors	
Frequent eye rubbing	9 (23.07%)
Regular eyedrop use	8 (20.51%)
Contact lens	11 (28.21%)
Touching of eyes after touching face mask	11 (28.21%)

HT, hypertension; DM, diabetes mellitus; Hx, history; PRN, as needed.

was 40 (range 19–72), with equal gender representation. Of note, most patients in our case series had mild to moderate COVID-19 disease (89.74%); 15.38% (6/39) reported symptoms suggestive of ocular surface inflammation, including epiphora (10.25%), itchiness (7.69%), and foreign body sensation (5.13%). However, only 1 patient (2.56%) had bilateral overt conjunctivitis, which began 2 days after onset of mild Upper Respiratory Tract Infection (URTI) symptoms and spontaneously resolved after 1 week. There was no correlation between the COVID-19 severity and ocular symptoms ($p = 0.308$).

All cases had conjunctival sampling for SARS-CoV-2 on day 1 postadmission and before hospital discharge (days 4–40). On average, the first swab was taken on day 6 since the onset of COVID-19 symptoms. The corresponding cycle threshold in matching nasopharyngeal samples was 29.81 on admission, which is indicative of intermediate to low viral load.[3] In all 78 conjunctival samples, SARS-CoV-2 was not detected by RT-PCR. This was true across the spectrum of COVID-19 disease severity.

Discussion

There are 2 possible explanations for viral nondetection in the conjunctiva in our study. Hypothetically, the various means of personal hygiene (Table 2) reported in our patients could partially account for the low transmission rate onto the conjunctiva surface both before and during the COVID-19 disease course (e.g., via self-inoculation). Because of the high level of vigilance in Hong Kong, the local residents have adopted stringent personal hygiene since early reports of COVID-19 in January 2020. This was reflected in our study participants, who had adopted universal face masking (100%), 4 times or more handwashings per day (100%), and daily mobile phone disinfection with alcohol wipes (76.92%). Other ocular protection included that 76.92% wore spectacles while 7.69% more wore goggles. Regarding risk factors of ocular transmission, 28.95% of study participants wore contact lenses, including the patient with conjunctivitis, who had worn daily disposable contact lenses.

On the other hand, the presence of active conjunctivitis symptoms without the detection of SARS-CoV-2 RNA on the conjunctiva may suggest ocular symptoms are a part of a systemic immunologic response instead of resulting from direct inoculation. There have been several reports on immunologic phenomenon associated with SARS-CoV-2, such as paediatric multisystem inflammatory syndrome,[4] Guillain-Barré syndrome,[5] and the observation of cytokine storm, where the imbalance between inflammatory innate response and impaired adaptive host immune defense led to systemic tissue damage and manifestations. However, if this hypothesis holds true, one would speculate that ocular involvements are likely to occur in COVID-19 patients with more severe disease systemically.

We believe that the simpler hypothesis of the ocular surface being infected by the virus (either via droplet transmission or via self-inoculation) is more likely. The risk of self-inoculation exists. As reported by a cross-sectional study of 534 patients, COVID-19 seldom presents initially with conjunctivitis (0.56%). In contrast, 4.12% developed conjunctivitis after systemic symptoms.[6] A COVID-19 case was also reported to develop follicular conjunctivitis with positive conjunctival carriage not at the beginning but late in the disease (day 13), amid antiviral treatments.[7] This raises the possibility that the virus is subsequently introduced into patients' eyes via self-inoculation. Therefore, good personal hygiene throughout the disease course would theoretically lower the chance of self-inoculation.

In summary, our study found that conjunctival carriage of SARS-CoV-2 is low even in place of standardised sampling technique. This is probably good news for ophthalmic health care workers. Nevertheless, adequate eye protection and stringent personal hygiene are still important as they may contribute to further reduce ocular COVID-19 transmission to minimal.

The present study is limited by the lack of a control group to assess the protective and risk factors of viral transmission of COVID-19 via the conjunctiva. Future case-controlled studies and the use of a validated personal hygiene questionnaire[8] would yield further fruitful findings because there are reports of positive conjunctiva swab for SARS-CoV-2 in the absence of ocular symptoms (Table 1) and vice versa.

The relationship between the presence of SARS-CoV-2 on the conjunctiva and ocular symptoms remains to be elucidated. In addition, molecular study also suggested the possibility of the cornea being an ocular site of infection instead of conjunctiva.[9] Future larger-scale studies aimed at investigating these issues could provide more insight.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcjo.2021.01.003.

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Footnotes and Disclosure

The authors have no proprietary or commercial interest in any materials discussed in this article.