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SARS-CoV-2 on Ocular Surfaces in a Cohort of Patients With COVID-19 From the Lombardy Region, Italy

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IMPORTANCE Since February 2020, coronavirus disease 2019 (COVID-19) has spread rapidly all over the world, with an epidemiological cluster in Lombardy, Italy. The viral communicability may be mediated by various body fluids, but insufficient information is available on the presence of the virus in human tears.

OBJECTIVES To investigate the rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in tears collected from patients with COVID-19 by means of real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) assay and to assess the association of virus presence with concomitant clinical conditions.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study conducted between April 9 and May 5, 2020. The setting was intensive care units at Azienda Socio-Sanitaria Territoriale (ASST) Sette-Laghi Hospital, University of Insubria, in Varese, Lombardy, Italy. A conjunctival swab was performed in 91 patients hospitalized for COVID-19, which was clinically diagnosed by rRT-PCR assay on nasopharyngeal swabs and by radiological imaging. Conjunctival swabs from 17 additional healthy volunteer participants with no symptoms of COVID-19 were examined to evaluate the availability and applicability of the conjunctival swab test.

EXPOSURE SARS-CoV-2 detection by means of rRT-PCR assay performed on the collected samples obtained by conjunctival swabs.

MAIN OUTCOMES AND MEASURES Conjunctival swab and nasopharyngeal swab results are reported, as well as demographic and clinical data.

RESULTS A total of 108 participants (mean [SD] age, 58.7 [14.2] years; 55 female and 53 male) were tested for SARS-CoV-2 using rRT-PCR assay, including 91 patients hospitalized with COVID-19 and 17 were healthy volunteers. SARS-CoV-2 was found on the ocular surface in 52 of 91 patients with COVID-19 (57.1%; 95% CI, 46.3%-67.5%), with a wide variability in the mean viral load from both eyes. Among a subset of 41 patients, concordance of 63.0% (95% CI, 41.0%-81.0%) was found between positive conjunctival and nasopharyngeal swab test results when performed within 2 days of each other. In 17 of these patients, nasopharyngeal swab results were negative for SARS-CoV-2. In 10 of these 17 patients, conjunctival swab results were positive for the virus.

CONCLUSIONS AND RELEVANCE In this study, SARS-CoV-2 RNA was found on the ocular surface in a large part of this cohort of patients with COVID-19, although the infectivity of this material could not be determined. Because patients may have positive test results with a conjunctival swab and negative results with a nasopharyngeal swab, use of the slightly invasive conjunctival swab may be considered as a supplementary diagnostic test.

Supplemental content

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In February 2020, coronavirus disease 2019 (COVID-19) associated with severe acute respiratory syndrome appeared in China[1-12] and rapidly spread all over the world, with an epidemiological cluster in Northern Italy.[9,13-17] Several reasons explain this rapid diffusion in the Lombardy region of Italy. The high population density with increased possibility of interpersonal contact, the prevalence of respiratory pathologies because of pollution, contact with the Chinese population for travel and business, and the nonwindy, temperate climate conditions promote the persistence of a virus in the environment. More than 90 000 citizens from the Lombardy region have officially been affected by the disease, with a high death toll reported.[18] The actual numbers are certainly much higher.

The etiological factor responsible for the disease has been identified in a new betacoronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus has high human transmission via airways but has a medium virulence.[19] Therefore, many people test positive for the presence of the virus without any signs of disease. SARS-CoV-2 RNA has been found in the nasopharyngeal tract and bronchial drainage,[1] in saliva,[20] in tears,[21-24] in urine,[25] and in feces[26] but not in seminal fluids.[27]

The objective of the present study was to use real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) analysis to investigate the presence of SARS-CoV-2 in tears collected from patients with COVID-19. We also aimed to assess the association of virus presence with concomitant systemic and local clinical conditions.

Key Points

Question What is the qualitative and quantitative presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the ocular surface in patients with coronavirus disease 2019 (COVID-19) hospitalized in intensive care units at a university hospital in Lombardy, Italy?

Findings Using reverse transcription–polymerase chain reaction assay, this study found that SARS-CoV-2 was present on the ocular surface in 52 of 91 patients with COVID-19 (57.1%). The virus may also be detected on ocular surfaces in patients with COVID-19 when the nasopharyngeal swab is negative.

Meaning These results suggest that SARS-CoV-2 may diffuse from ocular surfaces to the body.

Methods

Study Design

This cross-sectional study was conducted between April 9 and May 5, 2020, in intensive care units at Azienda Socio-Sanitaria Territoriale (ASST) Sette-Laghi Hospital, University of Insubria, in Varese, Lombardy, Italy. A conjunctival swab was performed in 2 cohorts (patients hospitalized for COVID-19 and healthy participants) and was examined by rRT-PCR assay to detect the presence of SARS-CoV-2. The study was carried out in accordance with the guidelines of the Declaration of Helsinki[28] and subsequent revisions and with the authorization of the Ethics Committee and Institutional Advisory Board of ATS (Agenzia per la Tutela della Salute) Insubria in Varese, Italy. Oral informed consent was obtained from study participants, who did not receive a stipend. All collected data were deidentified and protected by privacy safeguards. The study is registered on ClinicalTrials.gov (Identifier: NCT04402853).

We collected specimens and clinical data from 176 eyes of 91 patients hospitalized for COVID-19 in 3 different intensive care units (ICUs). Clinical diagnosis of COVID-19 disease was confirmed by nasopharyngeal swab positivity, symptoms of severe respiratory distress, characteristic chest imaging (radiograph and computed tomographic scan with ground glass opacifications), and lymphopenia.[5,8] All patients were from the central-northern area of Lombardy. We excluded patients with continuous positive airway pressure helmets or similar respiratory devices, as recommended by anesthesiologists for the safety of patients. We also collected specimens from 34 eyes of 17 healthy volunteer participants (10 women and 7 men) to evaluate the availability and applicability of the conjunctival swab test.

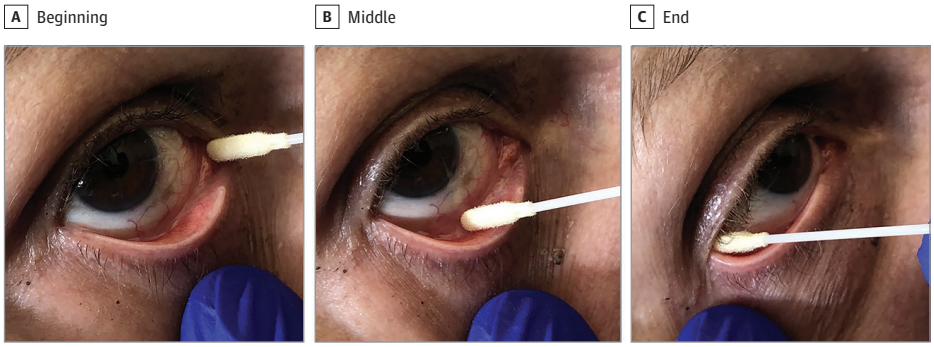
Before the conjunctival swab procedure, an ophthalmologist (E.P.) examined the status of eyelids, conjunctiva, and cornea. Eye examinations were done at the bedside without a slitlamp. Clinical information about hospitalization timing, results of diagnostic and serological examinations, and type of respiratory device was recorded using a smartphone during the procedure and later transcribed. We also documented results of the last nasopharyngeal swab for each patient.

The sampling procedure (**Figure 1**) was performed at the bedside by the same ophthalmologist (E.P.) in both eyes. The samples were obtained without topical anesthesia and after sufficient time had elapsed since lacrimal substitutes had been used. The conjunctival swabs were performed in the right eye first, and paired (right and left) conjunctival swabs were kept separate. A sample was available from only 1 eye in 6 patients (right eye in 4 patients and left eye in 2 patients) because of difficulties during the sampling procedure (lack of cooperation or technical problems). The conjunctival samples were absorbed by a dedicated swab with short fiber strands optimized for virus samples, with minimum patient discomfort (FLOQSwabs; COPAN, Brescia, Italy). Swabs for sampling of tears are shown in the eFigure in Supplement 1.

The conjunctival swab was then inserted into a dedicated vial with a transport fluid (UTM-RT-Hanks balanced salt solution enriched with proteins and sugars and with a neutral pH; COPAN). The use of this fluid ensures that the samples are preserved in ambient conditions for up to 48 hours. The vials were delivered to the laboratory within 45 minutes and stored at −80 °C after virus inactivation for 1 minute at 90 °C. The laboratory researcher (A.B.) then processed the samples within 2 days.

From the vial, 140 µL of each sample was subjected to RNA extraction (QIAmp viral RNA mini kit; QIAGEN) and eluted in 60 µL. One-step real-time polymerase chain reaction (PCR) was performed (Luna universal qPCR master mix; New England

Figure 1. Procedure for Sampling of Tears



A, First, the swab is leaned on the caruncula for 5 seconds. B, It is then slowly moved across the exposed inferior fornix conjunctiva to the external fornix (C) within 5 seconds. The samples were obtained without topical anesthesia and after sufficient time had elapsed since lacrimal substitutes had been used. In all patients, the conjunctival swabs were performed in the right eye first.

BioLabs) from 5 µL of extracted RNA. Forward (5′-ACCTTCCCAGGTAACAAACCA-3′) and reverse (5′-TTACCTTTCGGTCACACCCG-3′) primers targeting the 5′ untranslated region (5′UTR) of SARS-CoV-2 were used. Primers were designed using software (CLC Genomics Workbench; QIAGEN), and their specificity was checked using the BLAST database.[29]

Samples were run in 4 replicates together with a quantified positive control (SARS-CoV-2 RNA control; Twist Bioscience) on a PCR system (QuantStudio 5 real-time PCR; Thermo Fisher Scientific), with an annealing temperature of 60 °C. Provided at a concentration of 10^6 copies/µL, serial dilutions of RNA control from 10^4 to 10 copies/µL were used to construct a standard curve to perform an absolute quantification. Results were expressed as copies/µL (a positive viral load was defined as >50 copies/µL in at least 1 eye) according to the detection and amplification ability of the rRT-PCR instrument. In case of an uncertain result, an end point rRT-PCR and subsequent sequencing of the 5′UTR region were performed on the same RNA.

Retrotranscription, amplification, and sequence reaction were performed with a thermal cycler (Veriti; Perkin Elmer), and 251-base pair amplicon detection was performed with a chip (LabChipGx Touch24; Perkin Elmer). Obtained amplicons were sequenced by the Sanger method (SeqStudio genetic analyzer; Thermo Fisher Scientific). All collected specimens have been preserved in vials at −80 °C for future research.

Electronic medical records (eTable in Supplement 2) were uploaded and stored in a dedicated database. The system was provided by a medical platform in a data warehouse in Milan, Italy (Eumeda platform hosted by Aruba Business srl) to ensure data security and uninterrupted availability. The platform enabled efficient and immediate data visibility and rapid data extraction.

Statistical Analysis

Because of a lack of data regarding the prevalence of positive conjunctival swab results in the Italian COVID-19 population, there was no formal sample size calculation. Given the size of the reference population for the hospital and the epidemic trend curve at the start of the study, we expected to accrue approximately 100 patients. This size would enable estimation of the proportion of positive tests with a precision of about 10%.[30]

We summarized demographic and clinical features of eligible participants. Mean and standard proportions or absolute and relative frequencies were used for continuous and discrete variables, respectively. The same statistics were reported in patients with positive vs negative conjunctival swab results and were compared using *t* tests or χ^2 tests for continuous and discrete or dichotomous variables, respectively. Time between conjunctival swab performance and COVID-19 diagnosis was reported in weeks (range, 1-4 weeks).

We estimated the rate of patients with positive conjunctival swab results in the overall sample since COVID-19 diagnosis using the exact binomial distribution for 95% CIs. The same analyses were replicated for the latest available nasopharyngeal swab. In addition, we reported the prevalence of concordant-positive conjunctival swab results in both eyes. The viral load distribution variable was defined as the average between eyes in patients with concordant-positive results and as the viral load in the positive eye for individuals with positive conjunctival swab results in 1 eye only. We estimated the rate of both positive conjunctival and nasopharyngeal swabs, as well as both negative conjunctival and nasopharyngeal swabs, and the 95% CIs from the exact binomial distribution. The analyses were performed using SAS, 9.4 release (SAS Institute Inc), and graphics were drawn with R (The R Project for Statistical Computing). All *P* values were 2-sided but *P* values were not adjusted for multiple analyses.

Results

A total of 108 participants (mean [SD] age, 58.7 [14.2] years; 55 female and 53 male) were tested for SARS-CoV-2 using rRT-PCR assay. Ninety-one were hospitalized patients with COVID-19, and 17 were healthy participants. The mean (SD) age of the healthy participants was 49.5 (5.2) years. All conjunctival swabs were negative for SARS-CoV-2 among the healthy participants. The evaluation of the availability and applicability of the conjunctival swab test in these 17 showed that the procedure is annoying for the participant and easily repeatable by the operator.

Characteristics of the 91 hospitalized patients are shown in **Table 1**. Of these, 58 patients (63.7%) had positive naso-

Table 1. Demographic and Clinical Characteristics of 91 Patients Overall and by Conjunctival Swab Positivity or Negativity for SARS-CoV-2

Variable	No. (%)		
	All patients (N = 91)	Conjunctival swab positive (n = 52)[a]	Conjunctival swab negative (n = 39)
Age, mean (SD), y	67.9 (13.2)	68.3 (13.5)	67.3 (12.9)
Sex			
Female	45 (50.5)	27 (51.9)	18 (46.2)
Male	46 (49.5)	25 (48.1)	21 (53.8)
Nasopharyngeal swab test			
Positive	58 (63.7)	NA	NA
Negative	33 (36.3)	NA	NA
Comorbidities at COVID-19 diagnosis			
Cardiovascular diseases	53 (58.2)	31 (59.6)	22 (56.4)
Respiratory diseases	13 (14.3)	9 (17.3)	4 (10.3)
Autoimmune diseases	22 (24.2)	15 (28.8)	7 (17.9)
Neurological diseases	32 (35.2)	17 (32.7)	15 (38.5)
Endocrine diseases	65 (71.4)	38 (73.1)	27 (69.2)
Ocular diseases	5 (5.5)	2 (3.8)	3 (7.7)
Previous ocular surgery	3 (3.3)	2 (3.8)	1 (2.6)
Ocular signs[b]			
Hyperemia	3 (3.3)	3 (5.8)	0
Secretions	3 (3.3)	3 (5.8)	0
Blepharitis	5 (5.5)	4 (7.7)	1 (2.6)
Other signs	8 (8.8)	7 (13.5)	1 (2.6)
Hospitalization department			
Infectious diseases department	31 (34.1)	19 (36.5)	12 (30.8)
High intensity medicine department	42 (46.2)	24 (46.2)	18 (46.2)
Intensive care department	18 (19.8)	9 (17.3)	9 (23.1)
Respiratory devices at time of conjunctival swab[c]			
Ambient air	21 (23.1)	9 (17.3)	12 (30.8)
Cannulas	16 (17.6)	10 (19.2)	6 (15.4)
Venturi mask	30 (33.0)	19 (36.5)	11 (28.2)
Reservoir mask	13 (14.3)	8 (15.4)	5 (12.8)
Intubation	11 (12.1)	6 (11.5)	5 (12.8)

Abbreviations: COVID-19, coronavirus disease 2019; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a] A positive viral load was defined as greater than 50 copies/μL in at least 1 eye.

[b] Presence of signs or symptoms in at least 1 eye.

[c] Listed from least to greatest intensity (excluding patients with continuous positive airway pressure helmets or similar respiratory devices, as recommended by anesthesiologists for the safety of patients).

pharyngeal swab results and 33 (36.3%) had negative nasopharyngeal swab results.

SARS-CoV-2 was found on the ocular surface in 52 of the 91 patients hospitalized with COVID-19 (57.1%; 95% CI, 46.3%-67.5%). There was a wide variability in the average viral load from both eyes (median [range], 284 copies/μL [29-45 000 copies/μL]).

The virus was present in both eyes in 31 of 52 patients. Several patients (22 of 31 [71%]) had a slight difference in viral load values between the 2 eyes. A discrepancy in conjunctival swab results within the same patient (ie, 1 eye positive and 1 eye negative) was observed in 21 of 91 patients (23.1%); a viral load greater than 50 copies/μL was detected in 1 eye. The highest viral load of a single eye (up to 90 000 copies/μL) was found in patients with the virus detected in both eyes. The lowest value of viral load found in patients considered positive for the conjunctival swab (58 copies/μL) was found in patients in whom the virus was detected in 1 eye only.

No association was found between virus detection and comorbidities at COVID-19 diagnosis. A slightly higher prevalence of ocular signs or symptoms of surface inflammation in at least 1 eye was present among patients with positive conjunctival swab results than among those with negative conjunctival swab results. Neither the hospital department type nor the respiratory device at the time of conjunctival swab was associated with the presence of the virus on the ocular surface (Table 1).

Fifty-eight (63.7%; 95% CI, 53.0%-73.6%) of the hospitalized patients had positive nasopharyngeal swab results (**Table 2**). No differences in the number of positive conjunctival swabs were found among the 91 hospitalized patients according to time since COVID-19 diagnosis and clinical signs.

Forty-one patients had both nasopharyngeal and conjunctival swabs performed on the same day or within 2 days. A positive concordance of 63.0% (95% CI, 41.0%-81.0%) was found between conjunctival and nasopharyngeal swabs when performed within 2 days of each other. Among these 41 patients, only 7 of 17 patients (41.2%; 95% CI, 18.0%-67.0%) tested had negative conjunctival and nasopharyngeal swab results (**Table 3**). Again, 10 of these 17 patients had negative nasopharyngeal swab results but positive conjunctival swab results, with a mean viral load value of 881.7 copies/μL (range, 29-6900 copies/μL).

Table 2. Comparison of Patients With Positive Conjunctival Swab Results and Those With Positive Nasopharyngeal Swab Results[a]

Variable	Conjunctival swab positive		Nasopharyngeal swab positive	
	No.[b]/total No.[c]	% (95% CI)[d]	No.[e]/total No.[c]	% (95% CI)[d]
All patients	52/91	57.1 (46.3-67.5)	58/91	63.7 (53.0-73.6)
Time since COVID-19 diagnosis, wk				
1	14/26	53.9 (33.3-73.4)	NA	73.3 (58.1-85.4)
2	15/27	55.6 (35.3-74.5)	NA	82.4 (56.6-96.2)
3	10/14	71.4 (41.9-91.6)	NA	33.3 (0.1-70.1)
≥4	13/24	54.2 (32.8-74.5)	NA	40.0 (19.1-64.0)
χ^2 Test *P* value	NA	.71	NA	.005

Abbreviations: COVID-19, coronavirus disease 2019; NA, not applicable.

[a] A positive viral load was defined as greater than 50 copies/μL in at least 1 eye.

[b] Number of patients with positive conjunctival swab results.

[c] Number of patients with a COVID-19 diagnosis.

[d] The 95% CIs are from the exact binomial distribution.

[e] Number of patients with positive nasopharyngeal swab results.

Table 3. Results of Conjunctival and Nasopharyngeal Swab Tests Among 41 Patients With Both Tests Performed Within 2 Days

Variable	Nasopharyngeal swab positive		Nasopharyngeal swab negative	
	No.[a]/total No.[b]	Prevalence (95% CI)[c]	No.[d]/total No.[e]	Prevalence (95% CI)[c]
All patients	15/24	0.63 (0.41-0.81)	7/17	0.41 (0.18-0.67)
Time since COVID-19 diagnosis, wk				
1	7/8	0.88 (0.47-0.99)	2/6	0.33 (0.04-0.78)
≥2	8/16	0.50 (0.25-0.75)	5/11	0.45 (0.17-0.77)

Abbreviation: COVID-19, coronavirus disease 2019.

[a] Number of patients with positive conjunctival swab results.

[b] Number of patients in whom the results of the last nasopharyngeal swab were positive.

[c] The 95% CIs are from the exact binomial distribution.

[d] Number of patients with negative conjunctival swab results.

[e] Number of patients in whom the results of the last nasopharyngeal swab were negative.

Figure 2 shows the median values and 25th to 75th percentiles of viral load in 52 patients with positive conjunctival swab results. The median viral load was 1120 copies/μL for week 1, 303 copies/μL for week 2, 424 copies/μL for week 3, and 295 copies/μL for week 4. All 34 eyes of the 17 healthy control participants tested negative for SARS-CoV-2.

Discussion

In this study, SARS-CoV-2 was present on the ocular surface in patients with COVID-19 and was quantitatively and qualitatively detectable, but the infectivity of this material and thus the definitive clinical relevance could not be determined from this study. The high rates reported in this study (Tables 1 and 2) may be attributable to different reasons. The sample collections were performed by the same ophthalmologist following a precisely defined procedure and using a dedicated swab for molecular testing. Tear samples were processed in the same laboratory, which has extensive experience in processing thousands of body fluid samples a day from patients with COVID-19. Time between sample incubation and processing was minimized, as described in the Methods section, and a real-time PCR primer targeted to the 5′UTR region of SARS-CoV-2 was used in all cases.[20]

The few positive coronavirus conjunctival swab results reported in the literature[21-24,31-35] may be because of several critical issues in sampling and laboratory processes. First, the specimen collection procedures were not well explained in the articles and may be not reproducible. Second, samples were analyzed in various laboratories using different rRT-PCR procedures within the same study. Third, the tear samples may have been incubated with various fluids. Fourth, the interval from acquisition to processing was not stated, which may have altered the results if high. Fifth, knowledge about virus behavior and characteristics has increased in the last few months, with growing availability of detection methods.

There was a wide variability in the mean viral load from both eyes in the studied cohort. Several patients had a slight difference between the 2 eyes. A discrepancy in conjunctival swab results (ie, 1 eye positive and 1 eye negative) was observed in 21 of 91 patients (23.1%). The variability in viral load among the patients and the discrepancy between eyes might have different explanations. The presence of the virus on the eye surface could be variable (sometimes low or undetectable). In addition, the sampling procedure may be uncomfortable and poorly done, especially when performed in the second eye.

In comparison of conjunctival swab and nasopharyngeal swab results in 41 patients with COVID-19 when tests were performed within 2 days of each other, we observed a positive concordance of 63.0% (95% CI, 41.0%-81.0%) between the results of the tests (Table 3, left column). In the same subgroup, we observed that among the 17 patients with COVID-19 that had a negative nasopharyngeal swab test result, 10 patients had a positive conjunctival swab (Table 3, right column). These patients demonstrated a high viral load (approximate mean, 1000 copies/μL) in tear samples, pointing out that the virus was present in the body despite being undetectable in the nasopharyngeal tract. Studies[26,36] have

reported the presence of SARS-CoV-2 in various body fluids, but it was not always detectable in the nasopharyngeal tract in those cases.

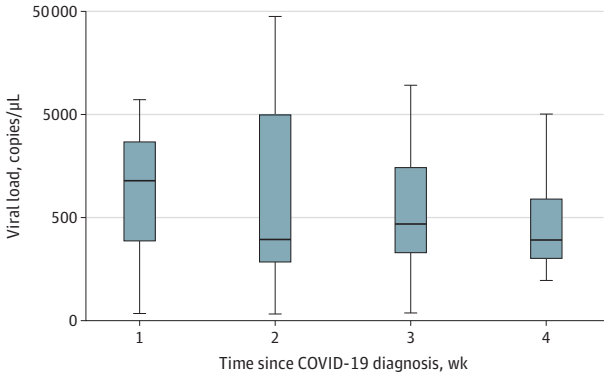
There could be many different explanations for the presence of the virus on the ocular surface, but these reasons are all speculative. For example, direct contagion from airborne droplets by infected people is possible, as well as particles diffused in the atmosphere.[1,9] Atmospheric particulates are known to function as carriers for many chemical and biological contaminants, including viruses.[37] By piggybacking, viruses adhere to atmospheric fine powders[38] consisting of solid or liquid particles that are able to remain in the atmosphere for hours, days, or longer, especially in a nonwindy and polluted climate like the Po Valley in Lombardy.[39] Increasing numbers of patients with COVID-19 have been diagnosed in this region since March 3, 2020, corresponding to excessive atmospheric particulate levels[40] recorded from February 10 to 29, 2020. This interval is recognized as the incubation period before clinical manifestation of the virus.

Regarding other means of viral diffusion into the eye, the literature reports direct contact with infected surfaces by the hands and transport to the mouth, nose, or other mucous membranes, such as the conjunctiva.[1,9,41,42] SARS-CoV-2 remains viable in aerosol form for hours even with a decreased infectious titer.[43] It may stay on various surfaces longer. For example, it can be detected on plastic for up to 72 hours, although with a great reduction in virus titer (ie, the virus could be viable on dry surfaces until 72 hours even if a reduction of <10-fold in titer is measured).[44] We speculate that the virus may diffuse in the fluid of the tears from lacrimal glands because of systemic viremia, as has been demonstrated for HIV.[45] Among the theories described herein, direct contagion from airborne droplets seems to be the most likely theory.

When SARS-CoV-2 infects the eyes from the conjunctiva, where airborne droplets land, the virus may diffuse into the body through the nasolacrimal duct, which is a pathway to the pharynx.[46-48] This contagion occurs despite the use of protective masks for the mouth and nose. The clinical case of the deceased ophthalmologist Dr Li Wenliang of Wuhan, China, described in the literature may be an example of such COVID-19 spread.[49] Although the infectivity of the viral material detected in the present study is unknown, these results support the use of eye protection for people working in environments where infection through the ocular route is feasible.[50-58] Eye protection probably should be considered if viral material might exceed certain limits, especially in the absence of wind or indoor systems designed to clear the air.

We evaluated the association between positive conjunctival swab results and the use of different respiratory devices[9] in patients with COVID-19, as considered in the eligibility criteria stated in the Methods section of our study. It is speculated that invasive maneuvers and respiratory devices like continuous positive airway pressure masks or helmets may increase the risk of viral diffusion by creating a closed environment around the head.[59] No associations were found between respiratory device use and conjunctival swab results. This finding indicates that respiratory devices may not be associated with viral diffusion of SARS-CoV-2 into tears (Table 1).

Figure 2. Viral Load in 52 Conjunctival Swab–Positive Patients According to Time Since COVID-19 Diagnosis



Shown are the median values and 25th to 75th percentiles of viral load. COVID-19 indicates coronavirus disease 2019.

We observed a low rate of ocular signs or symptoms in patients positive for the conjunctival swab (Table 1). A persistent palpebral edema could be secondary to the prone position of a patient for respiratory reasons, as we find in patients a few days after ocular surgery when a prone position is necessary.[60] However, data in the current literature are discordant about eye surface inflammatory involvement because many patients show different signs and symptoms, with difficult grading and evaluation.[61]

In respiratory airways, the infection and cellular entry of SARS-CoV-2 are mediated by the spike glycoprotein of coronavirus and the host cellular SARS-CoV receptor for angiotensin-converting enzyme 2 (ACE2).[62] Type II transmembrane serine protease (TMPRSS2) is required to promote SARS-CoV-2 entry by ACE2 cleavage to promote viral uptake.[63] However, the expression of ACE2 receptors in anterior ocular tissues, such as the conjunctiva or cornea, has yet to be established. More research exploring the hypothesis of SARS-CoV-2 ocular infection through ACE2 may be warranted. Various clinical signs of SARS-CoV-2 have been described in the literature,[64] but they appear to be nonspecific and different from viral conjunctivitis and keratitis-like adenovirus. Knowing that SARS-CoV-2 is present in the conjunctiva may represent future scenarios for immunological and body reaction therapy in these patients.

Regarding public health regulations, the current screening test for the detection of SARS-CoV-2 infection is rRT-PCR assay on respiratory specimens. This screening requires specific training for operators and is susceptible to false-negative results.[65] Detection tests using other body fluids are promising. Samples of tears and saliva are easily provided by the patients, they do not require specialized training for collection, and the procedure may not be uncomfortable.[20-22]

Limitations

This study has some limitations. We could not determine the infectivity of the viral material detected and thus the definitive clinical relevance. Other limitations include the cross-sectional design of the study, which lacks long-term prospective evaluation of the patients. The limited number of negative conjunctival swab in the presence of a positive nasopharyngeal test in patients with COVID-19 may be due to difficulties in patients' tears sampling and current overall limited knowledge. To evaluate the availability and applicability of the conjunctival swab test, we studied a group of 17 healthy volunteers with no symptoms or signs of COVID-19. All conjunctival swabs were negative for SARS-CoV-2 among these participants.

Conclusions

This cross-sectional study found that SARS-CoV-2 RNA was present on ocular surfaces in a large portion of the study cohort, although the infectivity of this material could not be determined from the study. Findings suggest that individuals with COVID-19 may test positive with a conjunctival swab and test negative with a nasopharyngeal swab. The slightly invasive conjunctival swab may be considered as a supplementary diagnostic test for COVID-19. Ongoing development of procedures and laboratory testing tools may improve the ability to investigate this use in the future.

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