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RESEARCH ARTICLE

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Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19?

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Abstract

The main purpose of this study is to evaluate the presence of viral RNA of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in conjunctival swab specimen of coronavirus disease 2019 (COVID-19) patients with and without conjunctivitis to establish the diagnostic value of reverse transcription-polymerase chain reaction (RT-PCR) in each case and to describe its clinical characteristics. A cross-sectional study was conducted at the Hospital Clinico San Carlos of Madrid, Spain. Thirty-six subjects from the COVID admission unit with laboratory-confirmed SARS-CoV-2 infection were included. Conjunctival swabs were collected from 18 patients with conjunctivitis and 18 patients without conjunctivitis and RT-PCR was performed. Conjunctival swab was collected from both eyes of 36 patients (72 eyes), detecting SARS-CoV-2 RNA in conjunctival swab of two patients (5.5%). Among the 18 patients with conjunctivitis, only one of them (5.5%) showed positive results. Likewise, SARS-CoV-2 RNA was detected in one patient without conjunctivitis (5.5%). The mean age of the 36 patients was 67.9 years (range, 28-92 years) and the male-to-female ratio was 0.44 (16:20). The mean days since the onset of COVID-19 symptoms until conjunctivitis manifestation was 8 (range, 1-24 days). The mean duration of the conjunctivitis was 3 days (range, 1-7 days). SARS-CoV-2 RNA may be detected in conjunctival swabs of both patients with and without conjunctivitis. This study revealed the same rate of positive results amongst the group with and without conjunctivitis, suggesting that detecting SARS-CoV-2 in ocular fluids is not conditioned on the presence of conjunctivitis. The presence of SARS-CoV-2 RNA in ocular samples highlights the role of the eye as a possible route of transmission of the disease.

KEYWORDS

conjunctivitis, coronavirus, COVID, diagnosis, PCR

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The disease has rapidly become a global health issue since it was first originated in China in December 2019.[1]

The main clinical features of COVID-19 are upper respiratory tract symptoms, myalgias, and diarrhea, but conjunctivitis has also been described as a clinical manifestation related to SARS-CoV-2 infection.[2,3] Evidence regarding the presence of SARS-CoV-2 RNA in tears and conjunctival secretions has been reported in patients with COVID-19.[4,5]

The main purpose of this study is to assess the presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of COVID-19 patients with and without conjunctivitis to establish the diagnostic value of reverse transcription-polymerase chain reaction (RT-PCR) in each case. Our secondary objective is to describe the clinical characteristics of conjunctivitis in a group of patients with laboratory-confirmed SARS-CoV-2 infection. The vast majority of studies published to date have been carried out in China. To the best of our knowledge, this is the first study of its kind in Europe.

2 | METHODS

This cross-sectional study was conducted at the Hospital Clinico San Carlos (HCSC) of Madrid, Spain, a tertiary referral hospital located in Madrid's metropolitan area. The study was approved by the Clinical Research Ethics Committee of this institution and was carried out in accordance with the tenets of the Declaration of Helsinki (Trial Registration Number 20/336_E_COVID). Informed consent was obtained from all patients.

Hospitalized patients for COVID-19 with and without conjunctivitis were consecutively recruited. The inclusion criteria were: over 18 years of age, positive RT-PCR test from nasopharyngeal swab for SARS-CoV-2, hospitalized due to COVID-19, and ability to give verbal consent. Critically ill patients and those unwilling or unable to give verbal consent were excluded.

For this study, a notification system was implemented for all health care personnel working at the COVID unit and evaluating the patients daily. Through this system, the ophthalmology department was notified daily of any new case of conjunctivitis amongst COVID-19 hospitalized patients. Cases reported as possible conjunctivitis were evaluated by two ophthalmologists during the first 24 hours after notification, and a conjunctival swab was collected from confirmed cases that also had positive RT-PCR from a nasopharyngeal swab. Consecutively, the sample from the patient's admitted to the following room number without conjunctivitis and confirmed SARS-CoV-2 infection was collected applying the same procedure. Both the examination and the sampling were carried out by the same physicians (N.G. and B.B.), following appropriate infection control and prevention measures. Patients with conjunctivitis were followed-up until resolution.

The conjunctival swab was collected from both eyes in every patient with a sterile synthetic fiber swab (Flexible Minitip Size Nylon Flocked Swab) into the lower fornix without topical anesthesia. We used the same swab to obtain a specimen from both eyes, first collecting the sample from the healthy eye in case of unilateral conjunctivitis. Caution was taken to avoid the possibility of sample contamination. The swab was immersed into a viral transport medium (Universal Transport Media; Copan, Italy), and stored at 4°C before being tested for SARS-CoV-2. RT-PCR assays were processed at the clinical microbiology laboratory of HCSC with quantitative GeneXpert Xpert Xpress SARS-CoV-2 (Cepheid). The cycle threshold (C_t) was measured.

The patient's age, sex, chest X-ray, laboratory test results (C-reactive protein), and days since onset of COVID-19 symptoms were obtained through the review of the patients' medical records. Also, clinical disease was classified as mild, moderate, severe, or critical based on CURB-65 score, physical examination, respiratory assessment (respiratory rate, dyspnea, blood oxygen saturation, and ventilation system requirements), or organ failure. As for the ophthalmological examination, the following variables were recorded: laterality, eye redness and discharge, duration of conjunctivitis, and treatment.

The main outcome measure is the overall proportion of positive RT-PCR test from conjunctival swab amongst COVID-19 in patients with and without conjunctivitis.

Regarding statistics, the overall prevalence results from the patients who tested positive for SARS-CoV-2 in the RT-PCR test from the conjunctival swab and it will be presented as a percentage of the total number of patients. The prevalence of positive RT-PCR among patients with conjunctivitis will be presented as a percentage of the total number of patients with conjunctivitis. The prevalence of positive RT-PCR among patients without conjunctivitis will also be presented as a percentage of the total number of patients without conjunctivitis. The distribution of sex, acute pneumonia, clinical severity, and the ophthalmological examination's findings will be presented as percentages. Likewise, the distribution of the quantitative covariates (age, C-reactive protein, days since onset of COVID-19 symptoms, and duration conjunctivitis) will be depicted through the median, first and third quartiles.

3 | RESULTS

A total of 36 patients were included in the study, 18 patients (50%) with conjunctivitis and 18 patients (50%) without conjunctivitis. Both groups had laboratory-confirmed COVID-19. Of the 689 hospitalized patients, 35 patients were reported as possible conjunctivitis, though 26 of them revealed a positive RT-PCR test from nasopharyngeal swab for SARS-CoV-2. Of those, 18 patients were finally diagnosed with conjunctivitis, three patients had a subconjunctival hemorrhage, two patients had a pterygium, one patient had eye redness related to antiglaucoma eye drops, one patient had a hordeolum, and one patient had pingueculitis. The conjunctival swab was collected from both eyes of the 36 patients included (72 eyes), detecting SARS-CoV-2 RNA in a conjunctival swab of two patients (5.5%). Among the 18 patients with conjunctivitis, one of them (5.5%) showed positive results for SARS-CoV-2 in the conjunctiva. Likewise, among the 18 COVID-19 patients without signs or symptoms of conjunctivitis, SARS-CoV-2 RNA was detected in one of them (5.5%).The mean age of the 36 patients was 67.9 years (range, 28-92 years) and the male-to-female ratio was 0.44 (16:20). Seventeen patients (47%) had mild, 12 patients (33%) had moderate disease, and seven patients (19%) had severe disease. Twenty-five of the patients (69.4%) presented pneumonia (Table 1).

The main clinical characteristics found on the patients with conjunctivitis are shown in Table 2. Half of them presented unilateral conjunctivitis and the other half were bilateral. Overall, 13 patients

TABLE 1 Clinical characteristics among patients with and without conjunctivitis

	With conjunctivitis	Without conjunctivitis
Sex		
Male (%)	39	50
Female (%)	61	50
Age, y	70.3 ± 21.6	65.4 ± 18.9
Clinical severity		
Mild (%)	50	44
Moderate (%)	33	33
Severe (%)	17	22
Pneumonia (%)	67	72

(72%) presented mild eye redness and in nine patients (50%), a moderate amount of secretions was observed. The mean days since onset of COVID-19 symptoms until conjunctivitis manifestation was 8 (range, 1-24 days). None of the patients showed conjunctival petechiae, corneal infiltrates, nor membranes or pseudomembranes. None of the patients experienced a decreased vision. The mean duration of the conjunctivitis was 3 days (range, 1-7 days).

The one patient with conjunctivitis and positive SARS-CoV-2 conjunctival swab results was a 92-year-old male classified as a severe case, but without pneumonia on chest X-ray. He presented conjunctivitis symptoms 5 days after onset of COVID-19 manifestations, which were fatigue, dizziness, and confusion. The C_t value measured in this patient was 25, which means an elevated viral load. In general, C_t levels are inversely proportional to the viral load.

Table 3 depicts the clinical characteristics of patients without conjunctivitis. Conjunctival swab samples from one patient without conjunctivitis yielded positive RT-PCR results. This patient was a 90-year-old male with multiple comorbidities, severe COVID-19, and compatible bilateral pneumonia on chest X-ray. The sample was collected 6 days after the onset of COVID-19 symptoms. Likewise, the C_t value found in this patient was 25.

4 | DISCUSSION

The novel coronavirus SARS-CoV-2 is an enveloped positive-sense RNA virus that is highly transmissible and has caused a huge global outbreak.[6] Despite the primary modes of transmission of SARS-CoV-2 infection are through respiratory droplets and contact with infected objects or surfaces, other modes of transmission,

TABLE 2 Clinical characteristics and findings in COVID-19 patients with conjunctivitis

Patient	Sex	Age, y	Clinical severity	Pneumonia	Laterality	Eye redness	Discharge	Days since onset of COVID symptoms	Duration of conjunctivitis	RT-PCR conjunctival swab
1	Female	84	2	+	unilateral	1+	1+	17	2	–
2	Male	75	3	+	bilateral	1+	2+	3	3	–
3	Male	82	2	+	bilateral	1+	2+	1	7	–
4	Female	40	1	+	bilateral	1+	1+	3	3	–
5	Female	33	3	+	bilateral	3+	1+	10	5	–
6	Female	81	1	+	unilateral	2+	2+	6	2	–
7	Female	87	2	+	unilateral	1+	1+	13	3	–
8	Male	92	3	–	bilateral	1+	2+	5	3	+
9	Male	91	2	+	bilateral	1+	2+	6	3	–
10	Female	88	1	+	unilateral	1+	2+	12	1	–
11	Female	92	1	–	unilateral	1+	2+	1	3	–
12	Female	81	1	–	unilateral	1+	1+	15	3	–
13	Female	38	1	+	unilateral	1+	2+	18	1	–
14	Female	91	2	+	unilateral	1+	2+	7	2	–
15	Male	43	1	–	bilateral	1+	1+	24	3	–
16	Male	62	1	–	bilateral	2+	1+	3	3	–
17	Male	43	2	+	bilateral	3+	1+	7	4	–
18	Female	63	1	–	unilateral	2+	1+	1	1	–

Note: 1—Mild; 2—moderate; and 3—severe.
Abbreviations: COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction.

TABLE 3 Clinical characteristics in COVID-19 patients without conjunctivitis

Patient	Sex	Age, y	Clinical severity	Pneumonia	Days since onset of COVID symptoms	RT-PCR conjunctival swab
1	Male	50	2	+	17	–
2	Female	63	1	+	19	–
3	Female	65	3	+	18	–
4	Female	79	1	–	8	–
5	Male	69	2	+	14	–
6	Male	90	3	+	6	+
7	Female	63	2	+	2	–
8	Male	78	3	+	16	–
9	Female	75	1	+	7	–
10	Male	35	3	+	20	–
11	Male	85	1	–	11	–
12	Female	78	2	+	6	–
13	Male	60	2	–	7	–
14	Female	28	1	+	12	–
15	Female	84	1	+	9	–
16	Male	63	1	–	3	–
17	Male	30	1	–	3	–
18	Female	82	2	+	5	–

Note: 1—Mild; 2—moderate; and 3—severe.
Abbreviations: COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction.

such as the ocular route, should not be overlooked, as SARS-CoV-2 RNA has been detected in tears and conjunctival secretions of patients with COVID-19.[4,5]

Previous reports have demonstrated that conjunctivitis is a clinical manifestation of COVID-19. Conjunctivitis may appear along with other COVID-19 symptoms, or may be the only presenting sign and symptom of the disease.[7] The reported prevalence of conjunctivitis varies widely among the different studies published at the time of writing this report. This prevalence ranges from 0.8%, reported by Guan et al[8] in a study that included 1099 patients with laboratory-confirmed COVID-19, to 31.6% in a case series that was also carried out in China.[3]

The natural history of the conjunctivitis in patients with COVID-19 seems to be a self-limiting conjunctivitis that improves in a few days without specific treatment. We did not find in our sample short-term complications associated to it, such as the presence of corneal infiltrates, membranes, or pseudomembranes. These characteristics differ to conjunctivitis of other etiologies, which has not been previously described.

Conjunctivitis has been associated with a more severe form of COVID-19. A recent meta-analysis showed that patients with severe COVID-19 infection had, at admission to the hospital, increased incidence of conjunctivitis.[9] These findings might have relevant clinical implications to recognize conjunctivitis as a possible sign related to a severe form of the disease. Nevertheless, our study found that only 17% of the patients with conjunctivitis had severe disease and 33% mild disease.

SARS-CoV-2 RNA has been detected in ocular fluids of patients with COVID-19 both with and without conjunctivitis.[10] However, collecting tears and ocular secretions for SARS-CoV-2 detection seem to provide a limited diagnostic value.[11] A recent study evaluated tears and conjunctival samples of 30 patients with confirmed novel coronavirus pneumonia. Of those, the only one patient with conjunctivitis revealed positive RT-PCR results.[4] Another report from Hubei providence, China, found positive results for SARS-CoV-2 on RT-PCR from both conjunctival and nasopharyngeal swabs of two patients with conjunctivitis.[2] In light of these results, it was initially suggested that the diagnostic value of the test might be greater in patients with conjunctivitis than in those without it. However, a more recent study that included 121 patients revealed that only one patient with conjunctivitis and two patients without conjunctivitis yielded positive RT-PCR results on conjunctival swab.[10] The proportion with positive results for conjunctival SARS-CoV-2 detection was 2.5% (3/121). Thus, the presence of SARS-CoV-2 RNA appears to be independent of the presence or absence of conjunctivitis associated with COVID-19. Our study included the same number of patients with and without conjunctivitis and laboratory-confirmed SARS-CoV-2 infection. Overall, our study revealed a proportion of

positive RT-PCR from conjunctival specimen of 5.5% (2/36), showing the same proportion of positive results among the conjunctivitis group and the group without conjunctivitis.

On the other hand, the two patients of our sample who tested positive for SARS-CoV-2 in conjunctival specimen were elderly people with severe forms of the disease. Likewise, the study previously mentioned by Zhou et al[10] found that two out of the three patients, that showed positive results in conjunctival swab, were classified as severe or critical cases. This brings out the possibility of detection SARS-CoV-2 in ocular secretions may be more likely in patients with severe disease. Because conjunctivitis has been associated with a more severe form of the disease, we could hypothesize that detecting SARS-CoV-2 in patients with conjunctivitis may be dependent of the severity of the disease, since both parameters appear to be interrelated.

Moreover, several studies have now established that the hyperinflammatory response induced by SARS-CoV-2 is a major cause of disease severity.[12] Thus, conjunctivitis in patients with COVID-19 could represent an inflammatory response of the disease, manifested by inflammation of the conjunctiva. The extent of the contribution of inflammation and the potential mechanisms responsible for this are still poorly understood.

PCR of nasopharyngeal specimen has demonstrated to be an effective method with overall high sensitivity and specificity for diagnosing novel coronavirus SARS-CoV-2. However, PCR essay of tears and conjunctival secretions appear to have a fairly low potential of detecting the virus, although this low positive rate of SARS-CoV-2 does not exclude the possibility of transmission of the infection through the ocular surface. Seah et al[13] evaluated the possibility of transmission through tears by assessing for the presence of SARS-CoV- 2 with viral isolation and RT-PCR analysis. A total of 64 samples were obtained during a 3 weeks period since the onset of symptoms. All samples showed negative results for SARS-CoV-2 on viral isolation and RT-PCR, suggesting that the risk of SARS-CoV-2 transmission through tears is low.

The timing of sample collection has been proposed as a factor to be considered when detecting the virus in ocular fluids. The mean days since onset of COVID-19 symptoms until sample collection were 10 days (range, 2-19). Standardized approaches for sample collection may yield more robust data about the persistence of the virus in the eye. Since most of the tears are drained into the inferior meatus of the nasal cavity, it may be possible that the virus rapidly passes from the eye surface to the respiratory system. Thus, SARS-CoV-2 would be present in the ocular surface for a limited time frame. Nevertheless, a case report by Chen et al[5] detected viral RNA in a patient with conjunctivitis for at least 5 days with the C_t values gradually increasing. Furthermore, a case report from Italy collected ocular swabs almost daily from a 65-year-old woman with conjunctivitis, detecting SARS-CoV-2 RNA for 18 consecutive days (from days 3 to 21 of the disease), and then 5 days after it became undetectable, the virus was detected again in the ocular swab sample collected at day 27.[14] These findings suggested sustained virus replication in the conjunctiva.

Hand-eye contact has been related with conjunctival congestion in patients with COVID-19. A study in 535 cases with COVID-19 found that hand-eye contact was independently correlated with conjunctival congestion.[15] Among the 27 cases with conjunctival congestion, 19 (70.4%) had a history of hand-eye contact, suggesting that frequent hand-eye contact may be a relevant risk factor for conjunctival congestion in patients with COVID-19, rather than the virus itself. The patient from our series with positive RT-PCR did not recall it, although most of the times inadvertently occurs.

This study had several limitations. First, this study includes a relatively small sample. Second, the sample was collected at different times of the disease in the different groups, which could affect the homogeneity of the results. Moreover, RT-PCR is a diagnostic test that does not possesses 100% sensitivity, so a negative test may represent a false negative result and do not rule out the presence of SARS-CoV-2. Both collecting different samples from each eye and collecting different samples over time may improve the sensitivity of the test. However, the saturation experienced by the health care system during this critical pandemic situation associated restrictions on access to patients, as well as limited resources for processing samples. Therefore, we were only able to collect one sample for both eyes from each patient. It would have been interesting to collect consecutive conjunctival specimens from those two patients who showed positive results to better understand the viral dynamics and quantify the C_t throughout the disease process.

In conclusion, SARS-CoV-2 RNA may be detected in tears and conjunctival swabs of both patients with and without conjunctivitis. Our study revealed the same rate with positive results amongst the group with and without conjunctivitis, suggesting that detecting SARS-CoV-2 in ocular fluids may not conditioned by the presence of conjunctivitis. Further studies are required to assess the risk of SARS-CoV-2 transmission through ocular secretions and the diagnostic value of RT-PCR in patients with and without conjunctivitis.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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