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Evaluation of Conjunctival Swab PCR Results in Patients with SARS-CoV-2 Infection

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ABSTRACT

Purpose: The study objective was to identify severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in conjunctival swabs from patients with confirmed SARS-CoV-2 infection.
Methods: Forty patients who tested positive by real-time reverse transcription (rRT)-polymerase chain reaction (PCR) of nasopharyngeal and oropharyngeal swabs were enrolled. Conjunctival swabbing was used to collect the tear and conjunctival secretions of patients.
Results: Conjunctival swab rRT-PCR was positive for three patients and negative for 37 patients. Ten of the patients (25%) were diagnosed with conjunctivitis during the ophthalmic examination. Of these patients, one was found positive by conjunctival swab rRT-PCR, and nine were found negative. The difference between patients who tested positive or negative using conjunctival swab rRT-PCR was without statistical significance in terms of the presence of conjunctivitis ($p = .720$).
Conclusion: The rate of positivity from conjunctival swab rRT-PCR was 7.5% in patients with confirmed SARS-CoV-2 infection.

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SARS-CoV-2; conjunctival swab; ocular disease

Coronaviridae is a well-known family of viruses that causes diseases ranging from the common cold with mild symptoms to potentially lethal human respiratory infections, such as Middle East respiratory syndrome and severe acute respiratory syndrome coronavirus (SARS-CoV).[1] New cases with pneumonia of unknown etiology were reported in Wuhan, China, in December 2019, and in January 2020, the disease pathogen was defined as the 2019-novel coronavirus.[2] It was later named "SARS-CoV-2" owing to its close similarity to SARS-CoV-1.[3]

The main transmission route of SARS-CoV-2 is close contact with infected people via respiratory droplets from coughing and sneezing. In addition, direct contact transmission occurs when a person touches a surface or object contaminated with the virus and subsequently touches his or her mouth, nose, or eyes.[4,5] The mucosa of the conjunctiva is linked to the upper respiratory tract. It is thought that the conjunctiva may be easily involved in SARS-CoV-2 infection and may act as a route of transmission.[6] In addition, the epithelium of the cornea and the conjunctiva contain angiotensin-converting enzyme 2 receptors. Therefore, they may play a major role in facilitating the entry of the virus into host cell membranes.[7] The identification of SARS-CoV-2 in conjunctival swabs has previously been evaluated.[8–10] Transmission via the conjunctival mucosa might be possible, and further studies are needed to determine this. Thus, the objective of the current study was to investigate the detection of SARS-CoV-2 using conjunctival swab real-time reverse transcription polymerase chain reaction (rRT-PCR) in patients with confirmed SARS-CoV-2 infection. In addition, the relationship between the ocular and laboratory findings was evaluated, along with any changes in relation to advancing age.

Materials and Methods

This prospective interventional case series study was conducted at Sakarya University Education and Research Hospital, in April 2020. Forty patients who tested positive by rRT-PCR of nasopharyngeal and oropharyngeal swabs, and who were previously diagnosed with SARS-CoV-2 infection, were enrolled in this prospective interventional case series study.

Low-dose chest computer tomography was performed when necessary. Systemic diseases, such as essential hypertension, diabetes mellitus, chronic obstructive pulmonary disease (COPD), and asthma, were investigated and noted. The findings of the laboratory investigations directed in the COVID-19 guide published by the Turkish Ministry of Health (i.e., D-dimer, C-reactive protein, urea, creatinine, ferritin, lactic dehydrogenase, lymphocytes, and neutrophils) were identified in the patients' records and noted.

An ophthalmic examination (using a penlight) of all patients was performed while they were hospitalized. The presence of conjunctivitis was noted. A history of previous ocular diseases was obtained from the patients and recorded.

Conjunctival swabs were taken within the first three days of hospitalization. Conjunctival swabbing was used to collect

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tear and conjunctival secretions from the patients. The lower eyelid of each patient was pulled down, and a disposable sampling swab was applied to cleanse the lower conjunctival fornix without anesthesia. The head of the sampling swab was inserted into the sample preservation solution in the disposable virus sample tube and was broken off the end of the swab along the red scale. Finally, the virus sampling tube was closed. Each patient's temperature was recorded at the time of the sample collection.

The specimens were stored at —40°C until rRT-PCR could be performed. The rRT-PCR assay was carried out using COVID-19 RT-PCR kit (PrimerDesign, United Kingdom) and Rotorgene Real-Time PCR System (Qiagen, Germany) as previously described.[11]

Statistical Analysis

Statistical analysis was carried out using SPSS® (version 23.0). Armonk, NY: IBM Corp.) Descriptive analysis was conducted to provide information on the general characteristics of the study population. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of the numerical variables was normal. The numeric variables were presented as the mean ± standard deviation. The independent *t*-test or the Mann-Whitney U test was employed to compare the numerical parameters. The categorical variables were assessed using the chi-square test. A *p*-value of < 0.050 was considered to represent statistical significance.

Ethical Approval

Prior approval to conduct the study was obtained from the relevant institutional review board, along with the informed written consent of the study participants. The study was conducted in accordance with the Declaration of Helsinki.

Results

The mean age of the 40 patients (15 females, 25 males) was 41.38 ± 23.72 years (a range of 1–82 years). Conjunctival swab rRT-PCR was positive for three patients and negative for 37 patients. The difference between patients who tested positive or negative in terms of mean age was without statistical significance (p = .870). Low-dose chest CT was performed in 35 patients.

An equal number of patients (two each) had asthma, COPD, and essential hypertension. None of them had diabetes mellitus. The difference between patients who tested positive or negative by conjunctival swab rRT-PCR was not statistically significant in terms of systemic disease (p = > 0.05 for all of them). Similarly, the difference in the laboratory findings between patients who tested positive or negative by conjunctival swab rRT-PCR was also not statistically significant. Table 1 depicts the laboratory results and the comparisons made in this regard. The mean body temperature of the patients was 36.90 ± 0.52°C; the difference between patients who tested positive or negative using conjunctival swab rRT-PCR was without statistical significance (p = .600).

Ten of the patients (25%) were diagnosed with clinical conjunctivitis during the ophthalmic examination. Of these patients, one was found positive by conjunctival swab rRT-PCR, and nine were found negative. The difference between patients who tested positive or negative using conjunctival swab rRT-PCR was without statistical significance with respect to the presence of conjunctivitis (p = .720). Of the 40 patients enrolled in this study, 14 had a history of previous ocular disorders. Refractive disorders, allergic conjunctivitis, and dry eye syndrome were present in 14, 8, and 5 patients, respectively. In addition, two patients had blepharoconjunctivitis, two had cataracts, and one had glaucoma.

Discussion

In the current study, of the 40 patients with confirmed SARS-CoV-2 infection, 3 (7.5%) tested positive using conjunctival swab rRT-PCR. In similar studies on patients with confirmed SARS-CoV-2 infection, Wu et al. reported a comparative positivity rate of 5.2% in 28 patients,[10] with figures of 1.3% in 72 patients and 6.6% by conjunctival swab rRT-PCR being cited by Zhang et al. and Xia et al., respectively.[9,12]

Table 1. Comparison of laboratory findings between patients with and without positive rRT-PCR tests from conjunctival swabs.

Variable	Conjunctival swabs Negative (n = 37)	Conjunctival swabs Positive (n = 3)	P value
Male/Female	23/14	2/1	.877
Mean age (years)	41.22 ± 24.19	43.33 ± 20.79	.877
Body temperature (°C)	36.90 ± 0.52	36.70 ± 0.30	.605
D-dimer (µg/L)	1768.67 ± 5,371.23	8413.33 ± 13,586.92	.317
CRP (mg/L)	40.29 ± 47.64	45.71 ± 37.00	.797
Albumin (g/L)	36.71 ± 5.09	31.80 ± 2.78	.062
Creatinine (mg/dL)	0.74 ± 0.34	0.76 ± 0.11	.725
Alanine aminotransferase (U/L)	35.94 ± 25.10	55.67 ± 39.63	.291
Aspartate aminotransferase (U/L)	38.57 ± 21.04	38.67 ± 18.50	.786
Serum ferritin (ng/mL)	474.47 ± 922.20	1024.01 ± 1,281.21	.426
Lactate dehydrogenase (U/L)	296.61 ± 121.43	380.00 ± 156.14	.288
Urea (mg/dL)	33.77 ± 19.04	32.33 ± 9.29	.569
White blood cell count (10^9/L)	6.20 ± 2.05	6.77 ± 4.93	.817
Lymphocyte count (10^9/L)	2.07 ± 1.31	2.17 ± 1.26	.778
Neutrophil count (10^9/L)	3.51 ± 1.74	3.81 ± 3.60	.898
Hemoglobine (g/L)	12.36 ± 1.36	11.70 ± 0.85	.280
Eosinophil count (10^9/L)	0.09 ± 0.09	0.12 ± 0.05	.208
Platelet count (10^9/L)	251.83 ± 109.96	276.67 ± 90.88	.397
Mean platelet volume (fL)	9.34 ± 1.63	9.84 ± 2.10	.521

The differences in the results in the literature might be elucidated by differences within the studies pertaining to, for example, the viral concentration in the samples, differences in the sampling time, and the speed with which the viruses were transported to the inferior nasal meatus.

Conjunctivitis was diagnosed in 10 (25%) patients in the current study. Only one of these patients tested positive using conjunctival swab rRT-PCR. In another large-scale study on 1,099 confirmed SARS-CoV-2 patients, conjunctival congestion was observed in 9 (1%) of the patients.[13]

In a recent study, 12 of 38 patients (32%) had ocular manifestations consistent with conjunctivitis, including conjunctival hyperemia, chemosis, epiphora, and increased secretions. Only two of the 12 patients tested positive by conjunctival swab rRT-PCR.[10] This situation may relate to the lower case numbers in our study. A conjunctivitis-induced inflammatory response suggests that it may relate to the death of infected cells. If we had identified conjunctivitis in the initial period, our RNA results might have been higher.

A comparison was made of the laboratory findings for D-dimer, C-reactive protein, urea, creatinine, ferritin, lactic dehydrogenase, lymphocytes, and neutrophils between the patients who tested positive or negative using conjuna largectival swab rRT-PCR, and the difference was not found to have statistical significance. Wu et al. reported that patients with conjunctivitis were more likely to have higher white blood cell and neutrophil counts and higher levels of procalcitonin, C-reactive protein, and lactate dehydrogenase than patients without ocular symptoms.[10] To the best of our knowledge, no other study has compared laboratory test results with conjunctival swab rRT-PCR results. The use of large sample size in future studies is needed to confirm the results of the current research.

In addition to investigating a correlation between the laboratory findings and positive conjunctival swab rRT-PCR results, the relationship with high body temperature was investigated in the current study. However, the difference was not found to have statistical significance. No evidence is available in the literature regarding this relationship. Wu et al. suggested that ocular symptoms commonly appeared in patients with severe pneumonia, but they did not experience high fever.[10]

In the current study, 14 patients (35%) had a previous history of ocular disorders. The limitation of this result was the lack of a control group. A real case-control study might give more reliable assessment about this item. On the other hand, Chen et al. retrospectively investigated 534 patients with SARS-CoV-2, and they identified conjunctivitis, dry eye syndrome, keratitis, cataracts, and a history of diabetic retinopathy in 85 patients; the rate of concomitant ocular disease was found to be 16%.[14] Ocular diseases frequently cause and increase the rate of hand–ocular surface contact time. Several studies have suggested that contact with tear secretions might be the principal mode of viral transmission.[15,16]

Concomitant eye diseases seem to have significant implications for the contact transmission route of SARS-CoV-2.

In the current study, the distribution of conjunctivitis, fever, and the laboratory findings was assessed in the patients according to their age, and the C-reactive protein, albumin, creatinine, urea, and lymphocyte levels were found to vary by age range. These markers tended to be impaired after the age of 20 years. It has already been shown that the severity of SARS-CoV-2 infection increases with age.[17] The results of the current study reflect this. The increase in the severity of SARS-CoV-2 infection might also increase with age as a consequence of increased social relationships and more risky contact.

The major limitation of this current study was vastly different sample sizes of groups with or without positive rRT-PCR test for SARS-CoV-2, from conjunctival swabs. In addition, there was not a control group, in this study. Another important limitation was that the rRT-PCR test for SARS-CoV-2 detected viral shedding not live virus. If live virus could be isolated with viral culture, it would be more reliable to assess the transmission route.

CONCLUSION

The rate of positivity from conjunctival swab rRT-PCR was 7.5% in patients with confirmed SARS-CoV-2 infection and 10% in patients with conjunctivitis. Patients with a history of previous ocular disease accounted for 35% of all patients with confirmed SARS-CoV-2 infection. However, the frequency of RNA positivity was minimal in patients who were COVID-19 positive. The ocular manifestations of SARS-CoV-2 infection appear to be important, and further studies are warranted in this regard.

Declaration of interest

The authors report no conflict of interest

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