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Research

Correlation of Conjunctival Swab PCR Positivity with Nasopharyngeal Swab PCR Positivity in COVID-19 Patients

COVİD-19 Hastalarında Nazofariengeal Sürüntü PCR Pozitifliğinin Konjonktival Sürüntü PCR Pozitifliği ile Korelasyonu

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ABSTRACT

Objective: To evaluate the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the tear and conjunctival secretions of laboratory-confirmed coronavirus disease-2019 (COVID-19) patients.

Methods: A total of 152 consecutive COVID-19 patients, confirmed by real-time polymerase chain reaction (RT-PCR) test in nasopharyngeal swabs, were included. The conjunctival swabs were taken from both eyes by the same ophthalmologist in the first 24-36 hours after positive test results for COVID-19 in the nasopharyngeal swabs were detected.

Results: Of the 152 patients, 96 (63.15%) were male and 56 (36.85%) were female. The mean age was 39.36±13.15 years. Sixteen (10.5%) patients had symptoms or findings such as tearing, stinging, redness, pain, and burning in their eyes. RT-PCR tests for SARS-CoV-2 RNA in conjunctival swabs were positive in 13 (8.55%) of 152 COVID-19 patients.

Conclusion: This study showed that SARS-CoV-2 may be found in the conjunctival swabs of laboratory-confirmed COVID-19 patients, and ocular secretions may be a possible route for virus transmission.

Keywords: Conjunctival swab, COVID-19, nasopharyngeal swab, RT-PCR, SARS-CoV-2

ÖZ

Amaç: Laboratuvar onaylı koronavirüs hastalığı-2019 (COVİD-19) hastalarının gözyaşı ve konjonktival sekresyonlarında şiddetli akut solunum sendromu koronavirüs 2 (SARS-CoV-2) varlığını değerlendirmektir.

Gereç ve Yöntem: Nazofaringeal sürüntüde gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) testi ile doğrulanan ardışık yüz elli iki COVİD-19 hastası dahil edildi. Nazofarengeal sürüntülerde COVİD-19 için pozitif test sonuçları tespit edildikten sonraki ilk 24-36 saat içinde aynı oftalmolog tarafından her iki gözden konjonktival sürüntüler alındı.

Bulgular: Yüz elli iki hastanın 96'sı (%63,15) erkek, 56'sı (%36,85) kadındı. Ortalama yaş 39,36±13,15 idi. On altı (%10,5) hastanın gözlerinde yaşarma, batma, kızarıklık, ağrı, yanma gibi semptom veya bulgular vardı. Konjonktival sürüntülerde SARS-CoV-2 RNA için RT-PCR testleri, 152 COVİD-19 hastasının 13'ünde (%8,55) pozitif tespit edildi.

Sonuç: Bu çalışma, laboratuvarca doğrulanmış COVİD-19 hastalarının konjonktival sürüntülerinde SARS-CoV-2'nin bulunabileceğini ve oküler sekresyonların virüs bulaşması için olası bir yol olabileceğini göstermiştir.

Anahtar Kelimeler: Konjonktival sürüntü, COVİD-19, nazofaringeal sürüntü, RT-PCR, SARS-CoV-2

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INTRODUCTION

Coronaviruses (CoV) of coronaviridae are single-strain ribonucleic acid (RNA) viruses that typically infect birds and mammals. These viruses usually cause mild common cold cases in humans. However, some rare CoV strains, such as Middle East respiratory syndrome CoV, severe acute respiratory syndrome coronavirus (SARS-CoV), and coronavirus disease-2019 (COVID-19), (2019-nCoV), known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), may cause respiratory failure, which has an increased mortality risk (1).

In December 2019, an outbreak of infectious lethal pneumonia caused by a new strain of CoV (SARS-CoV-2) (2) was announced in Wuhan, China. Hence, the disease is named COVID-19 or coronavirus disorder-2019. The disease soon became a major global health issue (3,4), and the World Health Organization declared a pandemic on March 11, 2020.

SARS-CoV-2 is highly contagious. It is mainly transmitted through the inhalation of droplets or aerosols released by an infected person or through contact routes with infected fomites (5). Ocular tissues are easily exposed to infectious droplets and fomites during close contact with infected persons and contaminated hands. This may cause the conjunctiva to be a direct target of infected droplets (6). The ocular surface may be a route to spread and limit the disease, which needs to be clarified. Ophthalmic examination, in particular, may be a risk factor for contracting SARS-CoV-2 infection because patients are examined in closer proximity by an ophthalmologist. Direct contact with the eyes of patients may be unavoidable during an ophthalmic examination.

There are an increasing number of studies on viral RNA detection in ocular secretions (7-10). In addition, some studies have demonstrated that the conjunctiva can be an important entry point for respiratory viruses (11) and that infected tears can flow into the nasopharynx and reach the lower respiratory tract (8-12). The presence of the virus in ocular secretions is important to understand the possible different modes of transmission of the virus.

This study aimed to evaluate the presence of viral RNA in tear and conjunctival secretions (swabs) of laboratory-confirmed COVID-19 patients via real-time polymerase chain reaction (RT-PCR).

METHODS

One hundred and fifty-two patients who were diagnosed with COVID-19 with a positive nasopharyngeal swab real-RT-PCR test (Bioeksen, Türkiye) and admitted to our clinic were included in this prospective, observational study. In this study, patients younger than 18 years and those receiving treatment for COVİD-19 were excluded.

The tear and conjunctival secretion samples were taken from both eyes of the patients by the same ophthalmologist in the first 24-36 hours after the nasopharyngeal swab RT-PCR was detected as positive. The lower eyelid was pulled down to expose the conjunctival sac, and tears and conjunctival samples were collected using disposable swabs (iClean) without topical anesthesia. This procedure was repeated in the other eye. The sampling swab was then placed inside the disposable virus sampling tube containing a protective solution. The marked upper end of the swab was cracked, and the virus sampling tube was sealed. The sterile medical gloves and other personal protective equipment were changed between patients and prevent possible infection and contamination. All samples were stored in a 4 °C refrigerator and sent to the laboratory for RT-PCR analysis with in 2 hours.

The study protocol was approved by the University of Health Sciences Türkiye, İstanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Researches Ethics Committee (decision no: 2020-12-33, date: 08.06.2020) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients included in the study.

RT-PCR Protocol

Detection of the species-specific RdRp (RNA dependent RNA polymerase) gene of COVID-19 was performed by genomic RNA isolation and subsequent qualitative RT-PCR. Tear samples of patients were collected in Biospeedy transfer tubes (Bioeksen, Türkiye) for genomic RNA isolation. The QIA Symphony DSP Virus/Pathogen kit and QIA Symphony isolation instrument (Qiagen, Germany) were used to obtain virus RNA from tear samples. A Biospeedy COVID-19 RT-qPCR detection kit (Bioeksen, Türkiye) was used in the qualitative RT-PCR study. The Bioeksen PCR Kit and Rotor-Gene Q Real-Time PCR instrument were used to provide RNA amplification for the isolated RNA samples. The CoV reaction setup and qPCR program are summarized in Table 1.

FAM/HEX (Patient channel/Internal control channel) channel proliferation curve images were examined for the interpretation of the results. Non-sigmoidal curves were considered negative. Quantitation cycle (Cq) was calculated. If Cq<38, the results were considered as positive and the results were interpreted according to Table 2.

Statistical Analysis

All data were analyzed using the Number Cruncher Statistical System (NCSS, Utah, USA). Data are described as means and standard deviations (mean ± SD), percentages, or medians. The distribution of the data was evaluated using the Kolmogorov-Smirnov test. Fisher-Freeman Halton: Pearson Q-square test and Fisher's Exact test were used to compare the qualitative data. P values 0.05 were considered statistically significant.

RESULTS

The demographics and defining characteristics of 152 COVID-19 patients and 13 patients (8.55%) with conjunctival swab PCR positive results are summarized in Table 3.

Defining characteristics include treatment types as inpatient or outpatient, existence of ocular complaints, and symptom status of the patients. There was no statistically significant difference between the treatment modalities of the patients whether they had eye complaints or whether they were asymptomatic or not, in terms of the presence of virus in the conjunctival swabs (Table 3).

One hundred and eleven (73.00%) of the 152 COVID-19-positive patients reported complaints (one or a few of fever, loss of taste and smell, chill, shivering, weakness, fatigue, cough, sore throat, back pain, and shortness of breath) at the time of admission to the hospital. Thirty-six (23.7%) patients had no complaint but had a history of contact with someone who was COVID-19 positive. Five (3.3%) patients were both asymptomatic and had no history of contact (Table 3). The demographics and defining characteristics of 13 conjunctival PCR-positive patients on a case-by-case basis are presented in Table 4. The status of PCR positivity of both samples taken from the nasopharynx and conjunctiva are also shown.

Two (15.4%) patients with positive conjunctival RT-PCR test were treated as inpatients with lung involvement. One patient had mild COVID-19 pneumonia and the other had moderate (intermediate) COVID-19 pneumonia radiologically. Four of the 11 outpatients underwent thorax computed tomography. Only one of the 4 patients who had thorax computed tomography had mild COVID-19 pneumonia findings.

In three patients, one or more of the following complaints were together: low back pain (lumbar pain), fever, cough, weakness, back pain, loss of taste and mell, and sore throat complaints (Table 4).

Table 1. Reaction setup and qPCR program

Reaction setup			qPCR program application protocol		
Component	**Addition order**	**Reaction**	**Cycle count**	**Temperature**	**Time**
2x prime script	1	10 µL	1	52 °C	5 min
Oligo mix	2	5 µL	1	95 °C	10 sec
Template nucleic acid	3	5 µL	40	95 °C	1 sec
Total reaction volume		20 µL		55 °C	30 sec
				FAM/HEX reading	

qPCR: Quantitative polymerase chain reaction

Table 2. Interpretation of SARS-CoV-2 results

Template	Non isolate		Positive control		Negative control		Interpretation
Target	**Wuhan RdRp**	**IC**	**Wuhan RdRp**	**IC**	**Wuhan RdRp**	**IC**	
State 1	Positive	Positive	Positive	Positive	Negative	Negative	SARS-CoV-2 positive
State 2	Negative	Positive	Positive	Positive	Negative	Negative	SARS-CoV-2 negative
State 3	Positive	Positive	Positive	Positive	Positive	Negative	Contamination: Re-experiment
State 4	Negative	Negative	Positive	Positive	Negative	Negative	Extraction/inhibition trouble: Dilute the nucleic acid isolate to 1/10, re-experiment.
State 5	Negative	Negative	Negative	Negative	Negative	Negative	Reactive trouble: The reagents are refreshed by contacting the supplier and re-experiment.

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, IC: Internal control, RdRp: RNA dependent RNA polymerase

Table 3. Distributions of defining characteristic

		All cases (n=152)	Conjunctival swab PCR (+) (n=13)	Conjunctival swab (-) PCR (+) (n=139)	p-value
Age (year)	Min-max (median)	19-72 (9.88)	21-59 (9.88)	19-72 (39)	
	Avr ± SD	39.29±13.19	42.15±12.01	39.61±13.41	
	19-40	92 (60.5)	-	92 (66.2)	[a]**0.001****
	41-60	49 (32.2)	7 (43.8)	42 (30.2)	
	>61	11 (7.2)	6 (46.2)	5 (3.6)	
Gender	Male	97 (63.8)	8 (61.5)	89 (64.0)	[b]**0.415**
	Female	55 (36.2)	5 (38.5)	50 (36.0)	
Treatment type	Inpatient	9 (5.9)	2 (15.4)	7 (5.1)	[c]**0.173**
	Outpatient	143 (94.1)	11 (84.6)	131(94.9)	
Ocular complaints	Existent	16 (10.5)	2 (15.4)	14 (10.1)	[c]**0.629**
	Non	136 (89.5)	11 (84.6)	125 (89.9)	
Patient complaints	Asymptomatic	41 (27.0)	4 (26.7)	37 (26.6)	[c]**0.749**
	Symptomatic	111 (73.0)	9 (73.3)	102 (73.4)	

[a]Fisher Freeman Halton test, [b]Pearson chi-square test, [c]Fisher Exact test,**p<0.01
Avr ± SD: Average ± standard deviation, min-max: Minimum-maximum, PCR: Polymerase chain reaction

Table 4. Positive conjunctival PCR tests

Patient no	Age	Gender	Patient complaint	Ocular complaint	Treatment type	PCR test	
						Nasopharynx	Conjunctiva
1	57	F	Low back pain (lumbar pain)	-	Outpatient	+	+
2	39	M	Fever, cough	-	Outpatient	+	+
3	43	F	Weakness, cough	-	Outpatient	+	+
4	39	M	Loss of taste and smell, weakness	+	Inpatient	+	+
5	59	F	Weakness, fatigue	-	Outpatient	+	+
6	31	M	Sore throat	-	Outpatient	+	+
7	32	M	Asymptomatic	-	Outpatient	+	+
8	52	F	Sore throat, cough, weakness	-	Outpatient	+	+
9	39	F	Cough, weakness, back pain	-	Inpatient	+	+
10	31	F	Asymptomatic	-	Outpatient	+	+
11	21	M	Asymptomatic	-	Outpatient	+	+
12	59	M	Muscle pain, cough	-	Outpatient	+	+
13	46	M	Asymptomatic	+	Outpatient	+	+

PCR: Polymerase chain reaction, M: Male, F: Female

DISCUSSION

SARS-CoV-2 is a highly contagious virus that is mainly spread by respiratory droplets, person-to-person contact (13) and contact with contaminated objects or surfaces. There is limited and controversial evidence of SARS-CoV-2 transmission by other routes.

It has been shown that the conjunctiva may be an infection source and the virus may be transmitted by the ocular route. Ocular inflammation findings related to COVID-19 were reported to have a prevalence ranging from 5% to 8% in some studies (14,15). There have been several studies on the isolation of SARS-CoV-2 from blood, saliva, and stool (16).

The ocular surface and conjunctiva are tissues in direct contact with the outdoor environment. Therefore, whether the new CoV will cause an eye infection or pass through the ocular surface is a issue that ophthalmologists focus on. It should not be ignored that SARS-CoV-2 can infect both the eyes and surrounding tissues. The virus may use ocular tissues as an extra route of transmission. However, the main reason is contamination of the conjunctival epithelium with infectious droplets and body fluids (6). In particular, hand–eye contact increases the risk of viral infection.

The enzymes angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) are believed to be the key proteins for entry of 2019-nCoV into host cells (17). Besides the studies indicating that ACE2, the main receptor for SARS-CoV-2, is not significantly expressed, a very low expression, in conjunctival samples of healthy and diseased people (18), there are studies showing that ACE2 receptors were located in non-pigmented epithelial cells of the ciliary body, corneal endothelial and epithelial cells, conjunctival epithelial cells, and trabecular meshwork cells in the anterior segment the expression of ACE2 in cornea and conjunctiva tissue of the eye (19). In addition, recent studies have reported the expression of ACE2 in the cornea and conjunctiva tissue of the eye (20). Studies have shown the overexpression of the ACE2 receptor gene in the human conjunctiva and cornea together with the TMPRSS2 protein (20,21). The presence of ACE2 and TMPRSS2 in the corneal limbal cells could explain the affinity of SARS-CoV-2 to this tissue and its existence in the tear (21). In agreement with the literatüre SARS-CoV nucleic acid can be detected in the ocular secretions of patients with SARS (7). Recently, Wuhan ophthalmologists detected viral nucleic acid in the conjunctival swab samples of laboratory-confirmed COVID-19 patients (22).

The possible infection of SARS-CoV-2 via the conjunctival route is a debated issue. Recent studies have reported that SARS-CoV-2 may be transmitted through mucous membranes, including the conjunctiva (23). These studies highlight the necessity for further research to investigate the possible transmission of SARS-CoV-2 through the conjunctival route, especially considering the recent findings that all COVID-19 patients have conjunctival congestion and ocular complaints (14,15). Ocular surface involvement characteristics include unilateral or bilateral bulbar conjunctival hyperemia, chemosis, follicular reaction of the palpebral conjunctiva, epiphora, and mild edema of the eyelids (24).

In a meta-analysis conducted by Sarma et al. (12) conjunctivitis findings were reported in only 3.75% of patients, and the positive rate for virus in the tear sample was found to be 1.949%. Zhang et al. (25) detected only 1 (0.9%) of the conjunctival swab samples of 102 COVID-19 patients (laboratory-confirmed 72 patients) with a positive result for the virus. In another study by Atum et al. (26) from 40 hospitalized COVID-19 patients, the PCR test of conjunctival swab samples collected in the first 3 days of their hospitalization was found to be positive for 3 (7.5%) of them. Mahmoud et al. (27) found a higher rate of SARS-CoV-2 RT-PCR positive tests in the conjunctival swab samples of COVİD-19 patients (8/28 patients-28.57%). Xia et al. (28) analyzed 2 conjunctival secretion samples taken at 2-3 days intervals after 7.33±3.82 days after the onset of symptoms from 30 patients with COVID-19. One of whom also complained of conjunctivitis. The SARS-CoV-2 RNA was found only in the tear and conjunctival secretion sample of a patient (3.3%) with conjunctivitis complaints. Li et al. (29) found that 4 patients (8.2%) were positive for SARS-CoV-2 RNA in a conjunctival swab sample by RT-PCR in their study, which included 49 COVID-19 patients without ocular symptoms. In contrast to those studies, in a study carried out by Deng et al. (30) including 114 COVID-19 patients, they found that no PCR test was positive for the virus in the conjunctival swab samples taken in 11±6.3 days.

In our study, 16 (10.52%) of 152 patients had ocular complaints. The RT-PCR test for SARS-CoV-2 was found to be positive in the conjunctival swab samples of 2 out of 16 patients. SARS-CoV-2 RNA was found in the conjunctival RT-PCR test in a total of 13 patients. Two of the 13 patients were among those with ocular complaints. The other 11 patients were among those without eye complaints. The conjunctival RT-PCR test was positive in 12.5% of COVID-19 patients with ocular complaints. Virus RNA was found in the conjunctival RT-PCR test in 11 (7.35%) of 136 patients without ocular complaints. Our results were similar to those of the previous studies mentioned above.

Briefly, in all these studies, the rate of patients with evidence of viral particles in the tear or conjunctival secretions remained between 0% and 7.5%, except for the study by Mahmoud et al. (27). They found it to be 28.57%, and this high rate may be due to various factors such as race difference, hygiene practices, and the fact that all of the included patients were inpatients.

However, conditions such as tests belonging to different manufacturers used in the studies, test techniques, patients included in the study (inpatient or outpatient, stil receiving treatment), sample sizes, differences in test sampling techniques, and intake times have an effect on the results of the studies.

In our study, the conjunctival swab samples were taken in the first 24 h after the nasopharyngeal RT-PCR tests were declared positive. Conjunctival swab samples were taken earlier than those in the aforementioned studies and from numerous patients before starting their treatment. Most of the patients were outpatients. We found an RT-PCR positivity rate of 8.55% in conjunctival swab samples from patients with confirmed diagnosis of SARS-CoV-2.

In regard to the present study results, COVID-19 RNA may be found in the conjunctival secretions and tears of COVID-19 patients with or without ocular symptoms, and the virus can be transmitted by the ocular route. The presence of SARS-CoV-2 RNA in the tear and conjunctival secretions was not associated with the presence of generalor ocular symptoms.

Various anatomical features indicate that the eye is a potential site for viral infection and a gateway for respiratory infection. The nasolacrimal system provides an important anatomical route between the ocular and respiratory systems (31). Therefore, the virus colonizing the conjunctiva can infect the upper and lower respiratory tracts.

This study has some limitations. First, only one conjunctival swab was obtained from each patient. The other limitation was that the patients did not know the first day of their complaints (some patients were also asymptomatic) during the RT-PCR test.

CONCLUSION

In agreement with some previous studies, we have detected that the conjunctival/eye secretions of COVID-19 patients, even if they are asymptomatic, may be a source of infection and transmission for medical professionals and other people. Ocular symptoms are rarely seen in COVID-19 patients. The eye may be not only a potential virus replication site but also an alternative route of virus transmission from the ocular surface to the respiratory and gastrointestinal tracts.

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ETHICS

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, İstanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Researches Ethics Committee (decision no: 2020-12-33, date: 08.06.2020) and adhered to the tenets of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all patients included in the study.

Authorship Contributions

Surgical and Medical Practices: M.Ö., Concept: M.Ö., Y.K., Ş.K., S.Z., K.K., Design: M.Ö., A.V., B.D., Ş.K., Ya.K., U.Y., K.K., Data Collection or Processing: M.Ö., A.V., B.D., Y.K., Ş.K., S.Z., K.K., Analysis or Interpretation: M.Ö., B.D., Y.K., Ş.K., U.Y., K.K., Literature Search: M.Ö., A.V., S.Z., Ya.K., Writing: M.Ö., A.V., B.D., U.Y.

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