

Coronavirus Pandemic

Detection of SARS-CoV-2 in the tears and conjunctival secretions of Coronavirus disease 2019 patients

Hüseyin Kaya¹, Ahmet Çalışkan², Mehmet Okul², Tuğba Sarı³, İsmail Hakkı Akbudak⁴

¹ Ophthalmology Department, Pamukkale University, Denizli, Turkey

² Department of Medical Microbiology, Pamukkale University, Denizli, Turkey

³ Department of Infectious Diseases and Clinical Microbiology, Pamukkale University, Denizli, Turkey

⁴ Department of Internal Medicine, Pamukkale University, Denizli, Turkey

Abstract

Introduction: Current studies suggest that tears and conjunctival secretions may be an important transmission route in coronavirus disease 2019 (COVID-19). The study aims to evaluate the presence of severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) virus in tears and conjunctival secretion of patients with COVID-19.

Methodology: A prospective interventional case series study was performed, and 32 patients with COVID-19 were selected at the Pamukkale University Hospital from 15 to 22 May 2020. The tear and conjunctival samples were collected by a conjunctival swab. Each specimen was sent to the laboratory for *reverse transcription-polymerase chain reaction* (RT-PCR) analyses. To avoid cross-infection, gloves and personal protective equipment were changed after collecting each sample.

Results: 32 patients (18 male, 14 female) with Covid-19 were included in this cross-sectional study. The average age of the patients was 52.81 ± 16.76 years. By the time of the first collection of conjunctival-tear samples, the mean time of the onset of complaints was 6.84 ± 6.81 (1-35) days. Tear-conjunctival samples from 5 patients (16%) without conjunctivitis yielded positive PCR results, 3 of whom had positive and 2 negative nasopharyngeal PCR results.

Conclusions: Five of 32 patients (16 %) without conjunctivitis or any eye symptoms had viral RNA in their tear-conjunctival samples. The possibility of transmission via tears and conjunctival secretions should be recognized even in the absence of conjunctivitis or other ocular manifestations.

Key words: COVID-19; SARS-CoV-2; tears; conjunctiva.

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Introduction

Coronaviruses are enveloped viruses containing a single-stranded RNA. Currently, different coronaviruses have been reported to cause infections in humans: HCoV-229E, -NL63, -OC43, and -HKU1, SARS-CoV, MERS-CoV, and 2019-nCoV. Coronaviruses can cause serious respiratory infections in humans [1,2]. For example, MERS-CoV was responsible for an outbreak of severe respiratory disease centered in the Middle East [3], while SARS-CoV was responsible for an outbreak of severe respiratory disease (SARS) in China and other countries during 2002–2003 [4]. Today, 2019-nCoV virus is responsible for a global COVID-19 pandemic.

The 2019 novel coronavirus disease (COVID-19) that started in China, in the city of Wuhan, has rapidly spread to all countries of the world and continues to seriously endanger patients' lives. At the time of writing,

the fast spread of the virus had caused 23,279,683 cases and 805,902 deaths globally [5]. So far, the COVID-19 pandemic has not been controlled and the number of cases worldwide is still rising. This may be because the transmission pathways of the coronavirus are very direct contact and respiratory droplets appear to be the major routes for COVID-19 infection [6]. On the other hand, other transmission paths such as of fecal-oral and aerosol, including through the ocular surface, have not been clearly demonstrated. Social isolation and personal protection are extremely important measures in the prevention and control of COVID-19. Whether the ocular surface is a way of spreading the disease is still an important issue that needs to be clarified.

Conjunctiva may be a direct inoculation site of infected droplets [7]. Conjunctiva, cornea or the epithelial cells of the nasolacrimal duct can take up virus from infected tears, or tears can drain to the

nasopharynx [8]. There are very few studies about detection of viral RNA in eye secretions. One study reported that the tears of SARS patients tested positive for viral nucleic acid, and that some patients only showed positive results in tear samples [9]. In another study, tear and conjunctival samples were collected from 20 suspected SARS patients, but SARS-CoV virus could not be detected by RT-PCR or isolated by viral culture [10]. A study of 114 patients with Covid-19 found negative conjunctival swab samples from all patients (none of whom had ocular symptoms) [11]. Another study on 17 patients could not demonstrate SARS-CoV-2 shedding in tears [12]. On the other hand, in a study evaluating 30 patients, SARS-CoV-2 was detected in the tears of one patient with conjunctivitis [13]. Two studies from China yielded positive PCR results from tear samples of Covid-19 patients [14,15]. In Iran, viral RNA was detected in tears of 3 out of 43 patients [16].

In our study, we aimed to evaluate the presence of viral RNA in the tear and conjunctival secretions of COVID-19 patients by reverse-transcription polymerase chain reaction (RT-PCR). Finding virus in tears and conjunctival secretions is important for understanding the potential diverse transmission routes for this virus.

Methodology

A prospective, cross-sectional interventional case series study was designed. The study protocol was approved by the Ethics Committee (date:12.05.2020, number:9). Written informed consent was obtained from all patients included in the study. Only confirmed novel coronavirus pneumonia (NCP) patients at the Hospital of Pamukkale University from 15 to 22 May 2020 were included in this study. NCP is defined as mild-moderate, or severe pneumonia.

Mild-moderate pneumonia was defined as a) symptoms such as fever, muscle/joint pain, cough and sore throat, respiratory rate < 30/minute, SpO₂ level > 90% at room air, and b) signs of mild to moderate pneumonia on chest radiography or tomography.

Severe pneumonia was defined as: a) symptoms such as fever, muscle/joint pain, cough and sore throat, tachypnea (> 30/minute), SpO₂ level ≤ 90% in room air, and b) bilateral diffuse pneumonia findings on chest radiography or tomography.

Patients' hospitalization criteria

Patients with positive test results

Those whose symptoms and findings improved were isolated at home until the 14th day following the

completion of the treatment period and improvement of the symptoms. Patients whose symptoms and findings continued or whose clinical condition deteriorated were hospitalized and taken to the hospital to evaluate the need for follow-up. It was then decided to continue monitoring at home or to admit to the hospital according to their clinical condition. Those with shortness of breath were taken to the hospital wearing a medical mask, either for a second sample, for hospitalization, or for other possible reasons.

Patients with negative test results

Those whose symptoms and signs improved were isolated at home until the 14th day following symptom improvement. Those whose symptoms and findings continued, who had fever, or increased coughing, or developed shortness of breath were taken to the hospital wearing a medical mask, either for a second sample, for hospitalization or for other possible reasons.

Patients were evaluated as mild, severe, and critical according to the clinical presentation based on the definitions in the "COVID-19 Diagnosis and Treatment Guide" published by the Turkish Ministry of Health. Mild illness is defined with characteristics such as fever, cough, sore throat, nasal congestion, and muscle or joint pain, with or without mild pneumonia together with a respiratory rate < 30/minute and an O₂ saturation above 90% while breathing room air. Severe illness is characterized by widespread findings of pneumonia in radiological examinations like chest radiography or computed tomography. Critical illness indicates a requirement for admission to the intensive care unit.

The conjunctival swab technique was used to collect tears and conjunctival samples from patients. The lower eyelid of each patient was opened and a disposable sampling swab was used to rub the conjunctiva of the lower eyelid fornix of the patient's eyes without anesthesia. The procedure was applied to both eyes. Next, the sampling swab was placed into preservation solution and the specimens were sent to the Pamukkale University laboratory for RT-PCR analysis. To avoid cross-infection, gloves and personal protective equipment were changed after collecting each sample.

At the time of collection of each tear-conjunctival sample, a nasopharyngeal sample was also acquired, and also sent to the laboratory for analysis. Before collecting the sample information was recorded about the time of onset of symptoms and how and when the antiviral drug was used for each patient. Favipiravir was applied in the treatment of the patients with pneumonia.

RT-PCR protocol

Bio-Speedy® COVID-19 RT-qPCR Detection Kit Version 2 (Bioeksan, Istanbul, Turkey) was used. VNAT (viral nucleic acid buffer) was used in the extraction kit. The kit was validated by the organizers of the European Centre for Disease Prevention and Control (ECDC) EVD-LabNet and ERLI-Net External Quality Assessment (EQA) for molecular detection of SARS-CoV-2. The RdRp gene targeted Wuhan-RdRp oligonucleotide set containing the 2019-nCoV Detection (RdRp gene) (FAM) and the internal control (IC) was the RNase P gene (HEX). The Prime Script Mix contained DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase, and ribonuclease inhibitor. The reaction was programmed into 45 cycles, 15 minutes 1 cycle at 45°C, 3 minutes 1 cycle at 95°C, 5 seconds at 95°C, and 35 seconds at 55°C.

1. 100 µL of sample was transferred to the tube containing 100 µL of viral nucleic acid buffer (vNAT).
2. The tube containing sample fluid and vNAT was vortexed for 10 seconds and nucleic acids were extracted.
3. For each sample, a mixed reaction solution containing 5 µL RdRp gene targeted Wuhan-RdRp oligonucleotide and 10 µL Prime Script Mix was prepared.
4. After mixing, 5 µL of fluid taken from the tube containing the sample and vNAT was transferred to the PCR tube containing 5 µL of oligonucleotide and 10 µL of prime.
5. PCR tubes containing 5 µL of extracted nucleic acid and 15 µL of mixed reaction solution were allowed to develop on the Qiagen Rotorgene Q device.
6. In approximately 90 minutes, the PCR procedure was completed and the proliferation curves obtained were examined. Non-sigmoidal curves were recorded as negative.
7. The threshold level was set to 0.02 for Qiagen Rotorgene Q. "Dynamic Tube" option was activated and the "Outlier Removal" option was set to 0.

8. The results were recorded as the number of threshold cycles 0.02 (Ct), the result was considered negative if Ct ≥ 40 and positive if Ct < 40.

Results

Thirty-two patients (18 males, 14 females) with Covid-19 were included in this cross-sectional study. The average age of the patients was 52.81 ± 16.76 years. At the time of first collection of conjunctival-tear samples, the mean time since onset was 6.84 ± 6.81(1-35) days. Conjunctivitis signs and symptoms were not observed in any patient included in the study. Five patients had mild, 22 patients had severe, and 5 patients had critical disease.

In 16 of 32 patients (50%), the nasopharyngeal RT-PCR sample was positive at the time that the conjunctival-tear sample was taken. The nasopharyngeal RT-PCR was negative in 16 patients at the time that the conjunctival-tear sample was taken. Of the 16 patients who were PCR negative at the time of sampling, 6 had previously been PCR positive and 10 were previously PCR negative.

Tear-conjunctival samples from 5 patients (16%) without conjunctivitis yielded positive PCR results; three of the patients had positive nasopharyngeal PCR and two had negative nasopharyngeal PCR results. The profile of the five cases with SARS-CoV-2 RNA detected in their tears are given in Table 1.

Discussion

In our study, we used conjunctival swabs to obtain tears and conjunctival secretions of 32 patients for standard RT-PCR assay. Five patients without any signs or symptoms of conjunctivitis were found to have viral RNA in their tear and conjunctival secretion samples.

Lu *et al.* reported that transmission of SARS-Cov-2 through eyes could be important and that the respiratory tract was probably not the only way for SARS-Cov-2 to be transmitted. They further recommended that all ophthalmologists who examined suspected cases should wear protective glasses [17]. Mucous

Table 1. Profiles of patients with PCR test positive for SARS-CoV-2.

Patients	1	2	3	4	5
Age	47	44	41	77	76
Sex	M	F	M	M	F
Clinical presentation and remarks	Critical	Severe, healthcare worker, HT, DM	Severe, healthcare worker	Critical, COPD	Severe, CKD
SARS-CoV-2 virus RNA from					
Tears-conjunctival	positive	positive	positive	positive	positive
Nasopharyngeal	negative	positive	positive	positive	negative
Day of collection from onset of symptoms	35	10	2	2	18

HT: hypertension; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease.

membranes are a transmission route for coronaviruses and unprotected eyes, mouth or nose were shown to increase the risk of SARS-Cov transmission [18]. Recent studies reported that ophthalmologists acquired SARS-Cov-2 accidentally during daily clinical practice. These results suggest that transmission could be connected to contact with tears or conjunctival secretions of patients [12].

Loon *et al.* reported on the detection of virus in tears and conjunctival secretions among SARS patients [9]. They tested tears and conjunctival secretions acquired from 36 suspected SARS patients by RT-PCR and found three positive results, although pharyngeal samples of one of those patients were negative. The researchers mentioned the possibility of contamination from the upper respiratory tract, and the presence of false negative RT-PCR results could not be ruled out [9]. Chan *et al.* tested conjunctival secretions from 17 confirmed SARS cases by RT-PCR and could not detect virus in these conjunctival secretions [10].

There are very few reports on detection of virus in tears and conjunctival fluid from in COVID-19 patients. Xia *et al.* collected tears, conjunctival secretions and pharyngeal samples twice from thirty COVID-19 patients and found only one patient with positive tear RT-PCR; that patient had conjunctivitis [13]. The authors speculated that conjunctiva and tears could act as a portal of entry for the virus [13]. Seah *et al.* found no positive results in tear and nasopharyngeal samples from 17 COVID-19 patients, collected between 3 and 20 days after initial symptom onset. The authors suggested that possible reasons for the failure to detect virus in these samples could be the absence of active conjunctivitis at time of sample collection, the small number of conjunctival secretion and tear samples, and the collection times [12].

In a study on 67 COVID-19 patients, conjunctival swab samples from one patient had a positive RT-PCR result, and two others had probable positive results [19]. A recent study included 38 COVID-19 patients, of which 12 were reported to have ocular manifestations, but only two of them had positive tear RT-PCR results [15]. Karimi *et al.* showed positive conjunctival swab sample results in three of 43 COVID-19 patients, of which one had conjunctivitis [16].

We found positive tear and conjunctival PCR results in 5 of 32 patients (16%), which is higher than the rates found in previous studies. This may be related to the technique of conjunctival swab removal, the expert taking the sample, and the patient's compliance, or to the sampling time. Previous studies have reported that viral load may be high in the early days of the

infection. The positive samples in our study were obtained from patients at different times after initiation of symptoms, from 2 to 35 days. Possibly, viral load and virus transmission to tears may vary from patient to patient. Also, contamination from the upper respiratory tract cannot be ruled out. That we found more positive samples might be related to the larger number of patients tested. In addition, racial variations or differences and variations in virus subtypes in different geographical regions may play a role.

In two of the five patients whose tear samples were positive, the result of the nasopharynx sample was negative. One of these patients, a 47-year-old male with critical disease, had had three previous negative results in the PCR analysis of nasopharyngeal and deep tracheal aspiration samples. However, the virus was isolated from his tear-conjunctival sample. Similarly, another patient with severe disease had negative results in previous nasopharyngeal samples, whereas in the tear samples we received, viruses could be detected. In such cases, collecting a tear-conjunctival sample at the same time as the nasopharynx sample may contribute to the diagnosis by increasing the probability of detecting the virus. Another feature of these two patients was that they were in the late phase of the disease (18th and 35th days), which shows that the virus can be detected from tears and conjunctiva even in the late period. Three out of five patients were in the stage of severe disease and two were in the stage of critical disease. This suggests that the severity of the disease and the amount of virus detectable in tears may be related.

The study had limitations. First, the sample size was relatively small. Second, only one sample of tear and conjunctival secretions were taken from each patient.

Conclusion

In conclusion, this study demonstrated a relatively high rate of SARSCoV-2 virus shedding in tears and conjunctival secretions, in 16% of 32 COVID-19 patients. Taking conjunctival swab samples may be beneficial for virus detection, especially for patients with a negative nasopharyngeal sample. The possibility of disease transmission through tears or conjunctival secretions should be taken into account even in the absence of conjunctivitis or any ocular manifestations.

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Corresponding author

Assist Prof. Hüseyin Kaya, MD
 Pamukkale University Hospital Ophthalmology Department
 Çamlaraltı district, 7
 20070 Kınıklı/ Denizli, Turkey
 Tel: 0902584440728 –5757
 Fax: 0902582131034
 Email: hsynkaya@gmail.com

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