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ORIGINAL PAPER

SARS-COV-2 viral load in tears of patients with COVID-19 in the early symptomatic stages: comparison of two different tear sampling methods

A. Sonmez · S. Aydın Kurna · F. G. Aslan · F. B. Kaplan · B. Açıkalın · P. Eker

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Abstract

Purpose To evaluate the presence of SARS-CoV-2 virus in tears of patients with COVID-19 in the early symptomatic stages and to compare two different sampling methods.

Materials and method In this cross-sectional study, tears sampling was performed in COVID-19 patients admitted within the first 7 days of symptom onset. The samples were collected with both conjunctival swabs and Schirmer strips. Each specimen was analyzed via RT-PCR. The viral load was evaluated in terms of the cycle threshold value. Ocular and systemic symptoms and comorbidities of the patients were also recorded.

Results Forty patients were included. The average time from the initiation of symptoms was 3.15 days. Unilateral conjunctivitis has been observed in 5% of patients and foreign body sensation in 7.5% of patients. No viral RNA was detected in the tear samples of the patients with ocular findings. The positivity rate for SARS-CoV-2 in tears was 2.5% ($n=1$). None of the samples collected by Schirmer test strips yielded positive polymerase chain reaction result for SARS-COV-2. The Ct value of the positive conjunctival swab was 36.03 and the nasopharyngeal Ct value of the same patient was 25.68.

Conclusion The SARS-CoV-2 viral shedding rate has been determined as 2.5% in the tears of early symptomatic stage COVID-19 patients. The viral load of the tears was lower than the naso-oropharynx. The conjunctival swab method is recommended in tear collection to evaluate the presence of SARS-CoV-2 by RT-PCR analysis in low viral load tears.

Keywords COVID-19 · SARS-CoV-2 · Tears · Viral load

Abbreviations

ACE	Angiotensin-converting enzyme
CRP	C reactive protein
COVID-19	Coronavirus disease 2019
LDH	Lactate dehydrogenase
NP	Naso-oropharyngeal

A. Sonmez (✉) · S. Aydın Kurna · F. B. Kaplan · B. Açıkalın
Department of Ophthalmology, Fatih Sultan Mehmet Training and Research Hospital, Istanbul, Turkey
e-mail: ademirciler@hotmail.com

S. Aydın Kurna
e-mail: sevdaydin@yahoo.com

F. B. Kaplan
e-mail: fthkaplan@gmail.com

B. Açıkalın
e-mail: banuoncel@superonline.com

F. G. Aslan · P. Eker
Presidency Central Laboratory, Istanbul Public Hospitals Services 2, Istanbul, Turkey
e-mail: ferhatgurkan33@hotmail.com

P. Eker
e-mail: pinareker@yahoo.com

RT-PCR	Reverse transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPSS	Statistical package for the social sciences
vNAT	Viral nucleic acid buffer tube
WHO	The World Health Organization

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel, enveloped RNA virüs and is a member of the beta-coronavirus family that have caused coronavirus disease 2019 (COVID-19) [1]. The pneumonia outbreak of COVID-19 has first been identified in Wuhan, China and due to the rapid spreading of cases, the World Health Organization (WHO) declared a pandemic on 03/11/2020 [2, 3]. SARS-CoV-2 is a highly contagious virus that is primarily transmitted through respiratory droplets and contact with infected individuals [4]. Lu et al. [5] reported that the disease can also be transmitted through the conjunctiva. SARS-CoV-2 gains entry into host cells through angiotensin-converting enzyme 2 (ACE2) via potential host receptors [6]. Recent studies elaborated that human conjunctival and corneal epithelium cells can also express ACE2 that provides a potential route for transocular entry and a possibility for COVID-19 [7, 8].

Fever and respiratory symptoms have been reported as the most common manifestations of the disease [9]. According to a systematic review, 11.64% of COVID-19 cases had some form of ocular symptoms [10] which could present itself as the first clinical manifestation [5]. SARS-CoV-2 has been detected in tear and conjunctival secretions both in the presence and absence of ocular symptoms, however, the reported prevalence of viral RNA varied [11–15]. This variation may be due to the discrepancies in sample collection timing, missing the period of virus shedding, collection technique, and small sample size. Ophthalmic evaluation involves direct contact with the patient's tear secretion and the potential for conjunctival transmission of SARS-CoV-2 is worth investigating, particularly for ophthalmologists.

In this study, we aimed to evaluate the presence of viral RNA in the tear and conjunctival secretions of COVID-19 patients using reverse transcription-polymerase chain reaction (RT-PCR) in the early stages of the disease and to compare two tear sampling methods.

Materıals and method

This prospective, cross-sectional study has been conducted in the University of Health Sciences Fatih Sultan Mehmet Training and Research Hospital between June 12, 2020 and October 27, 2020. The tear secretions of 40 patients clinically confirmed or suspected cases of COVID-19 disease have been investigated according to the definitions in the "COVID-19 Diagnosis and Treatment Guideline" published by the Turkish Ministry of Health. The study protocol was approved by the institutional ethics committee (date: 11.06.2020, Number: 44) and followed the Declaration of Helsinki. Written informed consent was obtained from all patients included in the study.

All of the laboratories confirmed patients had positive nasopharyngeal (NP) specimen of RT-PCR assay conducted within the last 24 h presenting at least one symptom of the disease (fever, cough or shortness of breath, muscle/joint pain, tiredness, headache, loss of sense of smell, and diarrhea). Clinically suspected cases were evaluated according to these data; (a) the presence of symptoms of COVID-19 disease (b) low or normal white blood cell count, (c) low lymphocyte count (d) high C-reactive protein (CRP), and/or lactate dehydrogenase (LDH), and/or D-dimer levels (e) computerized tomography lung imaging (unilateral or bilateral multilobar infiltration of the lungs of the peripheral zones and/or ground glass appearance). Tear samples were taken from all patients on the same day or within 24 h of collection of NP swabs. The patient's current temperature was recorded by the time of tear collection.

The baseline demographic parameters such as age, gender, the presence of comorbid diseases, the onset of symptoms, laboratory parameters, and NP PCR results were all recorded. The ocular findings were assessed by an external eye examination with a penlight. Asymptomatic patients, patients with symptom onset exceeding 7 days or that received treatment previously and individuals under the age of 18 were excluded from the study.

The clinical streamline of the patient has been classified as mild, moderate, or severe based on disease severity. The mild disease was defined as; cases with positive laboratory-confirmed results and any symptoms of COVID-19 disease (except dyspnea and tachypnea), but no pneumonia on chest tomography. The moderate disease was defined as; laboratory-confirmed or suspected patients with any symptoms of the disease, respiratory rate < 30/minute, SpO2 level > 90% in room air, and signs of mild to moderate pneumonia on chest tomography as confirmed by a radiologist. Severe disease was defined as; laboratory-confirmed or suspected patients with any of symptoms of the disease, tachypnea (> 30/min), SpO2 level ≤ 90% in room air, and bilateral diffuse pneumonia on chest tomography confirmed by a radiologist. All the patients were on the same systemic treatment protocol for COVID -19.

Tear sample collection

Tear samples were collected using disposable swabs and Schirmer paper strips by the same ophthalmologist on the posted days for COVID duty. No topical anesthesia was used during this procedure. Schirmer strips were folded and inserted into the lower lid of the eyes bilaterally, and after 3 min, the strips were removed and placed in a single viral nucleic acid buffer tube (vNAT). After retracting the lower eyelid, the inferior fornix was rubbed with a disposable nylon swab for 10 s. The conjunctival swabs from both eyes were placed in a single vNAT. To avoid cross-contamination, gloves were changed after collecting each sample and all the personal protective equipment was changed before moving on to the other patient. Tear specimens were stored at 4 °C and were immediately delivered to the laboratory for processing.

RT: PCR protocol

All the samples were placed in a vNAT (Bioeksen, Istanbul, Turkey) and delivered to the microbiology laboratory with a transport box adjustable at 4 °C without any delay. The samples were stored at − 20 °C until processing. They were extracted with the RINATM M14 automated nucleic acid extraction system in line with the manufacturer's recommendations. In the isolated samples, the presence of SARS-CoV-2 was investigated with one-step reverse transcription and real-time PCR with a commercial kit (Bio-Speedy, Bioeksen, Turkey), the SARS-CoV-2 Double Gene RT-qPCR kit, which targets the SARS-CoV-2 specific N and Orf1ab gene region. The results were recorded as the number of threshold cycles 0.05 (Ct), the result was considered negative if Ct ≥ 38 and positive if Ct < 38. The viral load was assessed in terms of the Ct value.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics 23 package (IBM SPSS, Turkey). The Shapiro–Wilk test was utilized to examine normal distribution. The data were obtained in the forms of mean, standard deviation, frequency, and percentage. The Kruskal–Wallis test was used for inter-group comparisons of parameters not showing a normal distribution. The Mann–Whitney *U* test was performed for comparisons of parameters between two groups not showing a normal distribution. A *p*-value of < 0.05 has been accepted as statistically significant.

Results

The gender distribution of the study population was 58% ($n = 23$) female and 42% ($n = 17$) were male. The mean age of the subjects was 51.3 ± 15.4 years (female 52.96 ± 14.07, male 49.05 ± 17.25). The average time from the initiation of symptoms was 3.15 days (1–6 days). All patients had at least one clinical feature; cough (45%), myalgia (37.5%), fatigue (35%), fever (30%), and dyspnea (27.5%) were defined as the most common symptoms. Other less frequent symptoms were diarrhea, sore throat, headache, and joint pain. Foreign body sensation has been observed in 7.5% ($n = 3$) patients and unilateral conjunctivitis in 5% ($n = 2$) patients, manifested by conjunctival congestion and mucus discharge at the time of admission.

A majority of the cases had laboratory-confirmed with COVİD-19 diagnosis as 87.5% ($n = 35$) and the remaining 12.5% ($n = 5$) were clinically suspected. The clinical presentation of the COVID-19 disease were mild in 25% ($n = 10$), moderate in 50% ($n = 20$) and severe in 25% ($n = 10$). One of the five patients with negative NP results was in the moderate group

and four were in the severe disease group. Fifty percent ($n=20$) of the patients had at least one systemic comorbid disease. The comorbidities were mainly hypertension (27.5%), diabetes mellitus (22.5%), and coronary artery disease (12.5%).

Mean Ct values for the mild, moderate, and severe disease were 24.43 ± 7.47, 24.20 ± 3.86, and 28.20 ± 2.28, respectively ($n=32$); Ct result was not evaluated in three patients whose NP samples were tested in a different laboratory. There was no statistically significant difference between the groups ($p=0.116$). The mean laboratory values of the patients were evaluated according to the severity of the disease and we have demonstrated that there is a negative correlation that was significant between disease severity with lymphocyte count and positive association with CRP and LDH values ($p<0.05$). Laboratory parameters and the Covid-19 severity scale of the patients are shown in Table 1.

All the patients with ocular findings had positive NP samples ($n=5$) while their tear results were negative. The positivity rate for SARS-CoV-2 in tears was 2.5% ($n=1$). Of 40 patients, RT-PCR showed positive results in the tears of one patient which was collected by conjunctival swabs. None of the samples collected by Schirmer test strips yielded positive polymerase chain reaction result for SARS-COV-2. The Ct value of the positive conjunctival swab was 36.03, and the naso-oropharyngeal Ct value of the same patient was 25.68 (Table 2).

Discussion

The patient population of this study consisted of 40 mild, moderate, and severe confirmed or suspected COVID-19 patients admitted to our hospital within the first 7 days of symptom onset. Previous reports

Table 1 Laboratory parameters and the Covid-19 severity scale of the patients

	COVID-19 severity scale				
	Mild	Moderate	Severe	Total	P[a]
Patients	10	20	10	40	
Age (Mean ± SD)	41.20 ± 14.09	51.90 ± 16.27	60.20 ± 8.36	51.3 ± 15.4	**0.009**
PCR positivity					
Patients	10	19	6	35	–
%	100	95	60	87.5	
Computerized tomography positivity					
Patients	0	20	10	30	–
%	0	100	100	75	
Cyclic threshold value					
Patients	10	16[b]	6	32	
Mean ± SD	24.43 ± 7.47	24.20 ± 3.86	28.20 ± 2.28	25.02 ± 5.16	0.116
Body temperature (Mean ± SD), °C	36.82 ± 0.57	36.98 ± 0.72	36.65 ± 0.49	36.9 ± 0.6	0.715
Oxygen Saturation (Mean ± SD), %	97.70 ± 0.82	97.2 ± 1.19	92.40 ± 2.31[c]	96.13 ± 2.62	**0.000**
Laboratory findings					
WBC Count (Mean ± SD), 10^3/µl	6.24 ± 0.70	6.42 ± 1.19	6.28 ± 1.87	6.34 ± 1.27	0.987
Lymphocyte Count (Mean ± SD), 10^3/µl	1.65 ± 0.49	1.92 ± 0.51	1.26 ± 0.64	1.68 ± 0.59	**0.021**
CRP (Mean ± SD), mg/dl	0.50 ± 0.50	3.92 ± 5.42	8.44 ± 4.50	4.20 ± 5.21	**0.000**
D dimer (Mean ± SD), ng/ml[d]	–	180.43 ± 474.94	330.05 ± 484.18	230.30 ± 475.07	0.202[e]
LDH (Mean ± SD), U/l	174.50 ± 69.70	233.30 ± 67.05	333.60 ± 97.79	243.68 ± 94.2	**0.001**

[a]Kruskal–Wallis Test

[b]Three patients cyclic threshold value could not be obtained

[c]All of the patients with severe disease had oxygen support

[d]D-dimer has only been studied in those with moderate to severe disease

[e]Mann–Whitney *U* Test

$p<0.05$ is considered statistically significant (bold reflected)

Table 2 Clinical findings, RT-PCR results and cycle threshold values of the cases

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
1	F	32	–	Mild	Myalgia, sore throat, headache, dyspnea	2	Positive	25.84	Negative	Negative	–	–	36.5	97	–	Normal
2	F	60	Hypertension	Mild	Sore throat, joint pain	1	Positive	31.6	Negative	Negative	–	–	36.5	98	–	Normal
3	M	69	Cardiac aritmia	Mild	Fatigue	3	Positive	33.93	Negative	Negative	–	–	36.5	98	–	Normal
4	M	27	–	Mild	Headache, joint pain	2	Positive	20.08	Negative	Negative	–	–	36.5	99	–	Normal
5	M	25	–	Mild	Sore throat	2	Positive	36.72	Negative	Negative	–	–	36.4	98	–	Normal
6	F	48	–	Moderate	Fatigue, diarrhea	2	Positive	25.01	Negative	Negative	–	–	36.5	99	–	Unilateral pneumonia
7	F	51	Asthma	Moderate	Dyspnea, fatigue	3	Positive	24.54	Negative	Negative	–	–	36.3	99	–	Bilateral pneumonia

Table 2 (continued)

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
8	F	43	–	Mild	Fever, myalgia, cough, dyspnea, diarrhea	4	Positive	18.39	Negative	Negative	–	–	37.7	96	–	Normal
9	M	45	–	Moderate	Fatigue, cough	3	Negative	–	Negative	Negative	–	–	36.5	99	–	Bilateral pneumonia
10	M	57	–	Severe	Cough, fatigue, dyspnea	3	Positive	26.62	Negative	Negative	–	–	36.5	93	+	Bilateral pneumonia
11	M	33	–	Mild	Fever, myalgia	4	Positive	22.46	Negative	Negative	–	–	36.5	98	–	Normal
12	M	58	Hypertension, diabetes mellitus, coronary artery disease	Severe	Fatigue, cough, fever	5	Negative	–	Negative	Negative	–	–	36.4	95	+	Unilateral pneumonia
13	F	45	–	Severe	Myalgia, fatigue, cough, dyspnea	6	Positive	28.08	Negative	Negative	–	–	36.9	88	+	Bilateral pneumonia

Table 2 (continued)

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
14	F	64	Diabetes mellitus, asthma	Severe	Myalgia, cough, fatigue	6	Negative	–	Negative	Negative	–	–	36.5	90	+	Bilateral pneumonia
15	M	52	Diabetes mellitus, hypertension	Moderate	Fever, cough, fatigue, myalgia	2	Positive	26.29	Negative	Negative	–	–	38.1	95	–	Bilateral pneumonia
16	F	51	Coronary artery disease	Moderate	Sore throat, myalgia, fever	2	Positive	22.84	Negative	Negative	–	–	36.4	98	–	Bilateral pneumonia
17	F	57	Diabetes mellitus, hypertension	Moderate	Cough, dyspnea, fever	4	Positive	UN	Negative	Negative	–	–	38.2	98	–	Unilateral pneumonia
18	M	22	–	Moderate	Fever, cough, dyspnea	4	Positive	31.09	Negative	Negative	–	Positive	38.1	97	–	Unilateral pneumonia
19	F	61	–	Moderate	Fatigue, cough	4	Positive	27.42	Negative	Negative	–	–	36.5	97	–	Bilateral pneumonia
20	F	36	–	Mild	Sore throat, joint pain	2	Positive	20.81	Negative	Negative	–	–	36.6	98	–	Normal

Table 2 (continued)

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
21	M	54	–	Severe	Dyspnea, fatigue	4	Positive	31.08	Negative	Negative	–	–	36.7	94	+	Bilateral pneumonia
22	F	33	–	Moderate	Fever, sore throat	3	Positive	15.83	Negative	Negative	–	Positive	37.8	97	–	Unilateral pneumonia
23	M	42	–	Mild	Fever, sore throat, diarrhea	2	Positive	21.33	Negative	Negative	–	–	37	97	–	Normal
24	F	45	–	Mild	Fever	2	Positive	13.21	Negative	Negative	–	–	38	98	–	Normal
25	F	33	–	Moderate	Fever, cough	3	Positive	19.0	Negative	Negative	–	–	38.3	96	–	Bilateral pneumonia
26	F	56	Diabetes mellitus, hypertension, coronary artery disease	Moderate	Cough, fatigue	3	Positive	20.63	Negative	Negative	–	–	36.4	97	–	Bilateral pneumonia
27	*M*	*63*	–	*Moderate*	*Sore throat, cough*	*4*	*Positive*	*25.68*	*Negative*	*Positive*	*36.03*	–	*36.4*	*97*	–	*Bilateral pneumonia*

Table 2 (continued)

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
28	F	65	Coronary artery disease, hypertension, chronic renal failure, cerebrovascular disease	Severe	Dyspnea, cough	2	Negative	-	Negative	Negative	–	–	36.6	93	+	Bilateral pneumonia
29	M	82	–	Moderate	Fatigue	4	Positive	28.46	Negative	Negative	–	–	37.4	96	–	Bilateral pneumonia
30	F	40	–	Moderate	Joint pain, myalgia, cough	3	Positive	26.7	Negative	Negative	–	–	36.5	97	–	Bilateral pneumonia
31	M	37	–	Moderate	Myalgia	2	Positive	20.13	Negative	Negative	–	–	36.5	99	–	Unilateral pneumonia
32	M	45	Hypertension	Moderate	Myalgia	2	Positive	22.72	Negative	Negative	–	–	36.7	96	–	Bilateral pneumonia
33	F	57	Diabetes mellitus	Moderate	Cough, sore throat, dyspnea	5	Positive	UN	Negative	Negative	–	–	36.5	98	–	Bilateral pneumonia

Table 2 (continued)

Case	Sex	Age, Y	Comorbidity	Severity Scale	Symptoms	Disease duration, days	Nasopharyngeal swab		Schirmer strips	Conjunctival swab		Conjunctivitis	Fever, °C	SpO_2, %	O_2 Support	CT scan
							RT-PCR	Cycle threshold N and Orf1ab gene	RT-PCR	RT-PCR	Cycle threshold N and Orf1ab gene					
34	F	56	Hypertension, asthma	Severe	Diarrhea	3	Negative	–	Negative	Negative	–	–	36	95	+	Bilateral pneumonia
35	F	59	Diabetes mellitus	Severe	Sore throat, myalgia, fever	2	Positive	26.53	Negative	Negative	–	–	37.8	93	+	Bilateral pneumonia
36	F	64	Hypertension diabetes mellitus	Moderate	Cough, myalgia	2	Positive	25.08	Negative	Negative	–	–	36.8	96	–	Bilateral pneumonia
37	M	74	Hypertension, coronary artery disease, cerebrovascular disease, diabetes mellitus	Severe	Dyspnea	3	Positive	25.93	Negative	Negative	–	–	36.9	90	+	Bilateral pneumonia
38	F	92	Hypertension	Moderate	Myalgia, fatigue	5	Positive	UN	Negative	Negative	–	–	36.5	97	–	Bilateral pneumonia
39	M	49	–	Moderate	Myalgia	3	Positive	25.81	Negative	Negative	–	–	37.2	96	–	Bilateral pneumonia
40	F	70	–	Severe	Cough, myalgia	5	Positive	30.93	Negative	Negative	–	–	36.2	93	+	Bilateral pneumonia

F Female, *M* Male, *UN* Unavailable

have focused on hospitalized patients. In order not to cause any selection bias, our cohort consisted of patients who were outpatient or hospitalized presented with the least symptom duration. Our study differs from the literature for having the patients in the early symptomatic period. Although there is no evidence yet of direct disease transmission through the tears, the possibility of nosocomial COVID-19 transmission in routine ophthalmic practice is of great concern.

In this study, 1 of 40 patients (2.5%) showed positive RT-PCR results in tears secretion collected by conjunctival swab. We did not detect any positivity in any of the tear samples taken by the Schirmer strips, including the patient with a positive conjunctival swab result. According to previous research, viral RNA detection rates in tear secretions have ranged from 0 to 24% [11–15]. Seah et al. utilized Schirmer strips to collect tear samples and performed consecutive sampling in patients but were unable to detect viral RNA in any of these samples. The majority of samples were collected in the second and third week of onset of symptoms and they did not classify the severity of the disease [11]. In an Indian study, positivity was detected in only 1 of 45 subjects (2.23%) similar to our study, however, this patient was from the asymptomatic patient group [12]. In a study from Wuhan 3 of 121 patients (2.4%) showed positive tear secretions; 2 of them were classified as severe or critical cases and another as mild to moderate [13]. The conjunctival samples were obtained from only one eye and the mean duration of disease was 15.0 ± 8.8 days. In order not to decrease diagnostic sensitivity with insufficient sample volume, we have collected the samples bilaterally and transferred them in a single vNAT [16].

In a study from Iran, Karimi et al. reported that tear samples with positive results were found in 3 of 30 (10%) patients. Their whole study population ($n=30$) was composed of severe laboratory-confirmed COVID-19 subjects with an average symptom duration of 3.27 (1–7 days) days [14]. Arora et al. [15] reported the comparison of different tear secretion collecting techniques on moderate (48%) and severe (52%) COVID-19 patients. The positivity rate of this study was 24% (18 in 75 patients); 14.7% ($n=11$) positive samples in the conjunctival swab group, and 9.3% ($n=7$) in the Schirmer strip group with an average symptom duration of 5 days (2–21 days). Zou et al. [17] reported that viral load decreased approximately 10 days after symptom onset. In our study, the average disease time on the day of admission was 3.15 days (1–6 days). To the best of our knowledge, this is the shortest mean symptom onset period in the published literature. The positivity rate of our study was significantly lower than the studies conducted in patients with short symptom duration time and moderate to severe disease activity [14, 15]

The prevalence of ocular manifestations was quite low in our analysis: Foreign body sensation has been observed in 7.5% ($n=3$) patients and unilateral conjunctivitis in 5% ($n=2$) patients. A meta-analysis of 3064 patients by Aggarwal et al. revealed that 11.64% (95% CI 5.54–17.75) of COVID-19 patients had some form of ocular symptoms; pain, foreign body sensation, conjunctival congestion, conjunctivitis, conjunctival chemosis, and itching (31.25%, 15.37%, 13.95%, 10.89%, 7%, 4.44%, and 6.55%, respectively) [10]. Zhou et al. reported ocular manifestations in 8 of 121 patients (6.6%); itching (62.5%), redness (37.5%), tearing (37.5%), discharge 25%), and foreign body sensation (25%). They reported three patients with positive tear sample results, only one patient showed ocular symptoms [13]. Karimi et al. published an article indicating that 2 of 43 patients showed ocular manifestations in the form of conjunctivitis (2.3%) and foreign body sensation (2.3%). They reported three patients with positive tear sample results; one patient with bilateral conjunctivitis and the other two patients with no ocular signs or symptoms [14]. Arora et al. [15] reported positive tear results in 18 of 75 patients (24%), without any ocular signs or symptoms. In our study, all patients with ocular findings had positive NP results while tear results were negative. There were no ocular manifestations in our patient with positive tear results.

SARS-CoV-2 has a central nervous system tropism and can cause multiple neurological manifestations that are neuroinvasive, including the eyes. Referring to experimental coronavirus retinopathy, Neri et al. hypothesized that it is a biphasic disease in which a direct viral insult underlies the infection and later progresses to a severe immune response leading to potentially massive tissue damage, which is a possible trigger for inflammation of both the retina and choroid. However, none of our patients complained of visual disturbances suggestive of uveitis or retinal pathology

[18, 19]. Vaccination is another potential source of ocular adverse events. Ocular findings occurring shortly after inactivated COVID-19 vaccination have been described [20]. The patients included in this study were not vaccinated.

The viral load of SARS-CoV-2 may be an important factor in determining both disease severity and the likelihood of transmission [21, 22]. The mean Ct value of the NP samples in our study was 25.02 ± 5.16 ($n = 32$); for the mild, moderate, and severe disease were 24.43 ± 7.47, 24.20 ± 3.86, and 28.20 ± 2.28, respectively, and no statistically significant difference between groups ($p = 0.116$) existed. In a study that Ct values of 875 Covid-19 patients were analyzed according to the disease severity; the median Ct value was 24 and a Ct value of < 25 indicated high viral load [23]. In addition to the low number of patients with severe disease in our study, five of these patients had negative NP PCR results. The Ct value of NP PCR tests of three patients with moderate disease severity could not be evaluated because they were processed in a different laboratory. It was reported that the viral load decreased during the second week of the disease [17, 24]. According to the hypothesis of the lacrimal duct as a viral conduit, we expected to detect more positive tear samples in symptomatic patients in the early stage of the disease, with high viral load. We detected only one positive conjunctival swab PCR test result in our patients. While the Ct value of this positive conjunctival swab was 36.03, the Ct value of the NP test performed the day before for the same patient was 25.68. Sample type is known to affect the Ct values and detected viral load [25]. According to our patient's polymerase chain reaction results, it could be claimed that the viral load of the nasopharynx is higher than the tear load. Bullard et al. reported that infectivity was significantly reduced when RT-PCR Ct values were greater than 24 and for every 1 unit increase in Ct decreased the risk of infectivity by 32% [26]. Patients with positive tear RT-PCR have a relatively lower viral load, suggesting a lower potential for transmission of infection through tears. Pro-inflammatory cytokines in tears may play a role in this discrepancy in viral load [27].

One of the objectives of this study was to compare two different tear sampling methods. The swab method has been used throughout the studies for tear collection and evaluation of viral RNA [12–15]. In a study in Singapore, multiple tear samples were collected using Schirmer paper strips from 17 cases between day 3 and 20 and none of the 64 RT-PCR reports were positive for SARS-CoV-2 RNA [11]. It was demonstrated that the viral load in the sample collected by the Schirmer strip was lower and also the ability to detect a sample with less viral load was greater with the conjunctival swab alone [15]. In our study, the viral load in the patients' tears, which was found to be lower than in the nasopharynx, could only be determined in the tear sample taken by conjunctival swab.

Positive nasopharyngeal RT-PCR detection of SARS-CoV-2 generally confirms the diagnosis COVID-19. However, false-negative RT-PCR from upper respiratory tract specimens is well documented [28]. We included mainly patients with positive RT-PCR (87.5%), but also hospitalized patients with suspected COVID -19 who had chest findings typical of viral pneumonia CT despite negative RT-PCR (12.5%). All patients with negative NP RT-PCR showed negative RT-PCR in both Schirmer smears and conjunctival swabs, which may explain the low rate of SARs-CoV-2 viruses in tears in this study.

Limitations of the study

The main limitation of this study can be elaborated as the small sample size, the inability to include the asymptomatic patients as the study center was an outbreak hospital, where symptomatic patients were predominantly directed. One other limitation can be stated as the failure to perform a direct slit-lamp examination, and one-time sampling. Additionally, since both gene regions were studied in the same channel, it could not be determined to which gene region the amplification curve belongs.

Conclusion

As a result, our findings reveal that the rate of SARS-CoV-2 viral shedding in tears is low in the early symptomatic stages of COVID-19. The viral load of the tears is lower than in the naso-oropharynx. However, further studies are required to better understand the mechanisms of the ocular transmission of SARS-CoV-2. The conjunctival swab method should be the

method of choice in tear collection to evaluate the presence of SARS-CoV-2 by RT-PCR analysis in low viral load tears.

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Data availability Data is available on request through the authors themselves.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study protocol was approved by the ethics committee of Health Sciences University Istanbul Fatih Sultan Mehmet Training and Research Hospital Clinical Research Ethics Committee (date: 11.06.2020, Number: 44). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

References

1. Zhu N, Zhang D, Wang W et al (2020) A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382(8):727–733. https://doi.org/10.1056/NEJMoa2001017
2. Wang C, Horby PW, Hayden FG, Gao GF (2020) A novel coronavirus outbreak of global health concern. Lancet 395(10223):470–473. https://doi.org/10.1016/S0140-6736(20)30185-9
3. World HealthOrganization (WHO). Novel Coronavirus (2019-nCoV) Situation Report—29 (18 February 2020). Geneva, Switzerland: World HealthOrganization; 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200218-sitrep-29-covid-19.pdf?sfvrsn=6262de9e_2
4. Li Q, Guan X, Wu P et al (2020) Early transmission dynamics in wuhan, china, of novel coronavirus-Infected pneumonia. N Engl J Med 382(13):1199–1207. https://doi.org/10.1056/NEJMoa2001316
5. Lu CW, Liu XF, Jia ZF (2020) 2019-nCoV transmission through the ocular surface must not be ignored. Lancet 395(10224):e39. https://doi.org/10.1016/S0140-6736(20)30313-5
6. Zhou P, Yang XL, Wang XG et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798):270–273. https://doi.org/10.1038/s41586-020-2012-7
7. Zhou L, Xu Z, Castiglione GM et al (2020) ACE2, and TMPRSS2 are expressed on the human ocular surface, suggesting susceptibility to SARS-CoV-2 infection. Ocul Surf 18(4):537–544. https://doi.org/10.1016/j.jtos.2020.06.007
8. Ma D, Chen CB, Jhanji V et al (2020) Expression of SARS-CoV-2 receptor ACE2 and TMPRSS2 in human primary conjunctival and pterygium cell lines and in mouse cornea. Eye 34(7):1212–1219. https://doi.org/10.1038/s41433-020-0939-4
9. McMichael TM, Currie DW, Clark S et al (2020) Epidemiology of Covid-19 in a long-term care facility in king County, Washington. N Engl J Med 382(21):2005–2011. https://doi.org/10.1056/NEJMoa2005412
10. Aggarwal K, Agarwal A, Jaiswal N et al (2020) Ocular surface manifestations of coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. PLoS One 15(11):e0241661. https://doi.org/10.1371/journal.pone.0241661
11. Seah IYJ, Anderson DE, Kang AEZ et al (2020) Assessing viral shedding and infectivity of tears in coronavirus disease 2019 (COVID-19) patients. Ophthalmology 127(7):977–979. https://doi.org/10.1016/j.ophtha.2020.03.026
12. Kumar K, Prakash AA, Gangasagara SB et al (2020) Presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of COVID-19 patients. Indian J Ophthalmol 68(6):1015–1017. https://doi.org/10.4103/ijo.IJO_1287_20
13. Zhou Y, Duan C, Zeng Y et al (2020) Ocular findings and proportion with conjunctival SARS-COV-2 in COVID-19 patients. Ophthalmology 127(7):982–983. https://doi.org/10.1016/j.ophtha.2020.04.028
14. Karimi S, Arabi A, Shahraki T, Safi S (2020) Detection of severe acute respiratory syndrome Coronavirus-2 in the tears of patients with Coronavirus disease 2019. Eye (Lond) 34(7):1220–1223. https://doi.org/10.1038/s41433-020-0965-2
15. Arora R, Goel R, Kumar S et al (2021) Evaluation of SARS-CoV-2 in tears of patients with moderate to severe COVID-19. Ophthalmology 128(4):494–503. https://doi.org/10.1016/j.ophtha.2020.08.029
16. Seitzman GD, Doan T (2020) No time for tears. Ophthalmology 127(7):980–981. https://doi.org/10.1016/j.ophtha.2020.03.030
17. Zou L, Ruan F, Huang M et al (2020) SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 382(12):1177–1179. https://doi.org/10.1056/NEJMc2001737
18. Neri P, Pichi F (2021) SARS-CoV-2 and the eye: the pandora's box of ocular immunology. J Ocul Pharmacol Ther 37(9):502–509. https://doi.org/10.1089/jop.2021.0058
19. Neri P, Pichi F (2020) COVID-19 and the eye immunity: lesson learned from the past and possible new therapeutic insights. Int Ophthalmol 40(5):1057–1060. https://doi.org/10.1007/s10792-020-01389-2
20. Pichi F, Aljneibi S, Neri P, Hay S, Dackiw C, Ghazi NG (2021) Association of ocular adverse events with inactivated COVID-19 vaccination in patients in Abu Dhabi. JAMA Ophthalmol 139(10):1131–1135. https://doi.org/10.1001/jamaophthalmol.2021.3477
21. Tom MR, Mina MJ (2020) To interpret the SARS-CoV-2 test, consider the cycle threshold value. Clin Infect Dis 71(16):2252–2254. https://doi.org/10.1093/cid/ciaa619
22. Joynt GM, Wu WK (2020) Understanding COVID-19: what does viral RNA load really mean? Lancet Infect Dis 20(6):635–636. https://doi.org/10.1016/S1473-3099(20)30237-1

23. Faíco-Filho KS, Passarelli VC, Bellei N (2020) Is higher viral load in SARS-CoV-2 associated with death? Am J Trop Med Hyg 103(5):2019–2021. https://doi.org/10.4269/ajtmh.20-0954
24. Huang JT, Ran RX, Lv ZH et al (2020) Chronological changes of viral shedding in adult inpatients With COVID-19 in Wuhan. China Clin Infect Dis 71(16):2158–2166. https://doi.org/10.1093/cid/ciaa631
25. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q (2020) Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 20(4):411–412. https://doi.org/10.1016/S1473-3099(20)30113-4
26. Bullard J, Dust K, Funk D et al (2020) Predicting infectious severe acute respiratory syndrome coronavirus 2 From diagnostic samples. Clin Infect Dis 71(10):2663–2666. https://doi.org/10.1093/cid/ciaa638
27. Neri P, Lamperti M, Pichi F (2020) SARS-COV-2, and eye immunity: the lesson was learned but we are not done yet: brainstorming on possible pathophysiology inspired by ocular models. Int Ophthalmol 40(8):1879–1883. https://doi.org/10.1007/s10792-020-01495-1
28. Fang Y, Zhang H, Xie J et al (2020) Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology 296(2):E115–E117. https://doi.org/10.1148/radiol.2020200432