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Evaluation of Severe Acute Respiratory Syndrome Coronavirus-2 RNA in tear specimens of hospitalised patients with confirmed novel Coronavirus disease 2019

Hastanede yatan yeni Coronavirus 2019 hastalarının gözyaşı örneklerinde Severe Acute Respiratory Syndrome Coronavirus-2 RNA'larının değerlendirilmesi

Nilüfer Zorlutuna Kaymak[1] Gizem Doğan Gökçe[2]
Mehmet Engin Tezcan[3] Serap Demir Tekol[4]

[1] Health Sciences University, Kartal Dr. Lütfi Kırdar City Hospital, Eye Clinic, Istanbul, Turkiye

[2] Balikesir Gonen State Hospital, Eye Clinic, Balikesir, Turkiye

[3] Health Sciences University, Kartal Dr. Lütfi Kırdar City Hospital, Department of Rheumatology, Istanbul, Turkiye

[4] Health Sciences University, Kartal Dr. Lütfi Kırdar City Hospital, Microbiology, Istanbul, Turkiye

ABSTRACT

Aim: We aimed to detect the presence of Severe Acute Respiratory Syndrome Coronavirus-2 RNA in the tears of patients with confirmed novel Coronavirus disease 2019.

Materials and Methods: This prospective study was performed at Health Science University Kartal Dr. Lutfi Kırdar City Hospital between 13-21 May 2020. Nasopharyngeal and tear samples of 15 patients with novel Coronavirus disease 2019 was performed for real-time polymerase chain reaction assay. Ocular and systemic signs and symptoms, chest computed tomographic scans, and results of laboratory blood tests as well as the drugs used for the treatment were noted and analyzed.

Results: Of the 15 patients nine (60%) were male and six (40%) were female. Mean age of the patients was 53.86±20.20 (21-89) years. Among them nine patients (60%) were positive for novel coronavirus disease 2019 on real-time polymerase chain reaction from nasopharyngeal specimens. None of the patients was positive for real-time polymerase chain reaction for Severe Acute Respiratory Syndrome Coronavirus-2 in tear specimens. Only two of 15 patients had bilateral conjunctivitis. These two patients had positive results for Severe Acute Respiratory Syndrome Coronavirus-2 on real-time polymerase chain reaction from nasopharyngeal swabs.

Conclusion: In this study Severe Acute Respiratory Syndrome Coronavirus-2 RNA could not be detected in the tear samples of the patients with confirmed novel Coronavirus disease 2019 with or without ocular symptoms and signs.

Keywords: COVID-19, SARS-CoV-2, conjunctivitis, ocular manifestations, RT-PCR.

ÖZ

Amaç: *Yeni Coronavirus 2019 hastalarının gözyaşlarında Severe Acute Respiratory Syndrome Coronavirus-2 RNA'sının varlığını tespit etmeyi amaçladık.*

Gereç ve Yöntem: *Bu prospektif çalışma Sağlık Bilimleri Üniversitesi Kartal Dr. Lütfi Kırdar Şehir Hastanesi'nde 13-21 Mayıs 2020 tarihleri arasında gerçekleştirildi. Real-time polimeraz zincir reaksiyonu testi için yeni Coronavirus 2019 hastalığı olan 15 hastanın nazofarengeal ve gözyaşı örnekleri alındı. Oküler ve sistemik belirti ve bulgular, göğüs bilgisayarlı tomografi taramaları ve tedavi için kullanılan ilaçların yanı sıra laboratuvar testlerinin sonuçları kaydedildi.*

Corresponding author: Nilüfer Zorlutuna Kaymak
Health Sciences University, Kartal Dr. Lütfi Kırdar City Hospital, Eye Clinic, Istanbul, Turkiye
E-mail: *n_zorlutuna@yahoo.com*
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Bulgular: *Onbeş hastanın dokuzu (60%) erkek, altısı (40%) kadındı. Hastaların ortalama yaşı 53.86±20.20 (21-89) idi. Dokuz hastanın (60%) nazofarengeal örneklerinde yeni Coronavirus 2019 hastalığı için real-time polimeraz zincir reaksiyon testi pozitifti. Hiçbir hastanın gözyaşı örneğinde Severe Acute Respiratory Syndrome Coronavirus-2 real-time polimeraz zincir reaksiyon testi pozitif değildi. Onbeş hastanın sadece ikisinde bilateral konjonktivit mevcuttu. Bu iki hastanın nazofarengeal örneklerinde Severe Acute Respiratory Syndrome Coronavirus-2 real-time polimeraz zincir reaksiyon testinin sonucu pozitifti.*

Sonuç: *Bu çalışmada oküler belirti ve bulgusu olsun ya da olmasın yeni Coronavirus 2019 hastalarının gözyaşı örneklerinde Severe Acute Respiratory Syndrome Coronavirus-2 RNA tespit edilememiştir.*

Anahtar Sözcükler: *COVID-19, SARS-CoV-2, konjonktivit, oküler bulgular, RT-PCR.*

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes novel Coronavirus Disease 2019 (COVID-19). The disease quickly spread throughout the world after it originated in Wuhan, China in December 2019 (1, 2). On 11th March 2020, World Health Organization (WHO) declared the disease a pandemic (3). After initial emerge in China, travel related cases started appearing from all over the world. The first case in Turkey was recorded on March 11, 2020. At the time of writing this study 148, 399, 311 people have been infected by the virus globally and there have been 3, 131, 077 deaths. In Turkey these numbers are 4,667, 281 and 38.711 respectively (4). The COVID-19 pandemic has not been controlled and continues to threaten the human-life in all over the world. The disease still doesn't have an effective treatment and the vaccination program conducted in the world have not reached the sufficient number of people; therefore understanding the transmission routes and preventing the transmission of the disease remains the only and crucial way of preventing the disease.

The main route of transmission is known to be through contact of mucous membranes of the eyes, nose and mouth with respiratory droplets or fomites. Viral RNA has been found in the nasopharyngeal tract, saliva, tears, urine and feces of the patients (5). Transmission via airborne respiratory droplets are well-recognized. The other ways of transmission have been discussed in many studies, but have not been elucidated yet.

The aim of our study was to evaluate the presence of SARS-CoV-2 RNA in tears of patients with confirmed COVID-19 and to describe the clinical spectrum of ocular and systemic symptoms together with nasopharyngeal and tear swab samples.

MATERIALS and METHODS

This prospective interventionally study was performed at a tertiary COVID-19 pandemic clinic. Fifteen patients with confirmed COVID-19, with or without ocular symptoms who were hospitalized at Kartal Dr. Lütfi Kırdar City Hospital between 13th and 21st May 2020 were included in the study. Suspect and probable cases and critically ill patients were excluded. The clinical diagnosis of COVID-19 were made through two different approaches. The patients with positive real-time polimeraz chain reaction (RT-PCR) for SARS-COV-2 were accepted as COVID-19 or the patients with negative RT-PCR got COVID-19 diagnosis, if they fullfilled all three clinical criteria including having fever and/or respiratory symptoms, compatible chest imaging findings and decreased lymphocyte count with normal or decreased white blood cell count. The disease was classified as mild and severe. Patients with mild pneumonia, oxygen saturation between 90-94% on room air and ≥24 breaths/minute respiratory rate were diagnosed as having mild disease while patients with pneumonia and at least one of the followings including: oxygen saturation <90% on room air, >30 breaths/minute respiratory rate, severe respiratory distress were diagnosed as having severe disease. Nasopharyngeal and tear samples were taken on the first day of admission from all patients. Before collecting the first samples, we recorded the time of onset of symptoms and the antiviral drugs used for each patient. A single ophthalmologist (GDG) posted for COVID duty collected both samples. Nasopharyngeal samples were taken according to World Health Organization (WHO) protocol. The tear specimens were taken randomly from one eye of the patients without ocular symptoms. In patients having ocular symptoms tears samples were taken from the affected eye if one eye is affected or from any of the affected eyes randomly if both eyes are affected. Tear samples were collected with sterile swabs placed in the lower fornix for ten seconds. We didn't use topical anesthesia during the procedure. The tips of the swab sticks were broken off and put into a viral transport media kit (VTM- Innomed). All necessary precautions were taken in order to prevent the spread of infection. Nazopharyngeal and tear specimens were stored

at 4 ℃ and sent to Kartal Dr. Lütfi Kırdar City Hospital laboratory as soon as possible. Viral RNA was extracted from all samples with High Pure Viral RNA Kit (Roche Life Science) by following instructions as described in manufacturer's protocol. Extracted RNA was tested by RT-PCR targeting SARS-CoV-2-specific RdRp (RNA-dependent RNA polymerase) gene fragment (Biospeedy, SARS-CoV-2 qPCR Detection Kit). RT-PCR analyses was performed with CFX96 Touch RT-PCR Detection System (Bio-Rad).

This study was approved by both Kartal Dr. Lütfi Kırdar City Hospital Local Research Ethics Committee and Turkish Health Ministry. It was conducted accordingly to the Helsinki Declaration. Signed informed consent was obtained from patients before the collection of each samples.

RESULTS

Fifteen patients were evaluated with an average age of 53.86±20.20 (21-89) years. Out of 15 patients nine (60%) were male and six (40%) were female. Nasopharyngeal RT-PCR samples were positive in nine (60%) of the 15 patients and negative in six (40%) of the 15 patients. Tear samples were negative in all of the 15 patients. The mean time of the onset of the systemic symptoms by the time of sampling was 2.53±2.21 (1-8) days. The average day for hospitalization was 6.73±3.65 (2-15) days. Two patients had Alzheimer disease. Because of the impaired mental functions of these patients, a detailed history could not be obtained about their symptoms. At the time of sampling the most common systemic symptoms were coughing in eight patients (53.33%), dyspne in eight patients (53.33%), fatique in six (40.00%) patients, and fever in four (26.66%) patients. The other symptoms were arthralgia, headache, diarrhea, nausea and loss of appetite. Two of 15 patients had ocular manifestations consistent with bilateral conjunctivits including redness, itching and burring. One of the patients with conjunctivitis had Alzheimer disease. These two patients had positive RT-PCR from nasopharyngeal swabs. None of our patients experienced ocular symptoms as the first manifestation of COVID-19. Among 15 patients nine (60%) had moderete disease while six (40%) patients had severe. None of them were critically ill. Demographic features, clinical profiles and laboratory results of the patients are shown in Table-1.

DISCUSSION

SARS-CoV-2 may occur at the ocular surface by direct inoculation of the conjunctiva from infectious droplets, transition of the virus through the nasolacrimal duct from the respiratory tract or the lacrimal gland infection and viral shedding (6). Also the affinity of SARS-COVs to angiotensin converting enzyme-2 (ACE-2) which is known to be the cell receptor for these viruses makes them possible to infect ocular structures. The human conjunctiva and cornea express ACE-2 receptor, theoretically which SARS-CoV-2 can bind and cause infection (7).

Recently, some studies have been conducted to detect the virus on the ocular surface of patients with COVID-19. The studies detecting SARS-CoV-2 nucleotides in tear films of patients with COVID-19 show different positivity rate ranging from 0% to 57.1% (8-19). In our study, we used conjunctival swabs to obtain tear samples of 15 COVID-19 patients and we could not show the presence of viral RNA in tears of these patients. In the study of Deng et al. on 114 patients, all of the ocular samples were negative (9). Similiarly, Seah et al. could not detect viral RNA in tear samples of their patients (8). Different from our study they collected a total of 64 tear samples from 17 patient's both eyes with one week intervals. They used Schirmer strip to obtain the samples. All tear samples were negative while naspharyngeal swab samples continued to show positive results. They claimed that the role of the lacrimal duct as a passageway for the virus may be wrong.

On the other hand some studies report positive SARS-CoV-2 RT-PCR results in tear samples of COVID-19 patients. In a study including 30 patients Xia et al. collected two samples from each patient at 2-3 days interval. Two conjunctival samples from one patient who also had conjunctivitis yielded positive (3.3%) (11). Zhang et al. reported that among 72 patients confirmed by SARS-CoV-2 RT-PCR, two patients had conjunctivitis and SARS-CoV-2 RT-PCR were positive in ocular samples in one of these patients (1.3%) (12).

On the contrary Kaya et al., Arora et al. and Azzolini et al. reported a relatively higher positive rates of 16%, 24% and 57.1% respectively (10, 13, 14). Different from other studies Arora et al. collected tear samples via conjunctival swab plus Schirmer's test strips, only conjunctival swab and only Schirmer's test strips in group 1, 2 and 3 with positive results of 11 (14.7%), 11 (14.7%) and 7 (9.3%) patients in these groups respectively. They took samples from both eyes. 18 (24%) of 75 patients and 29 (12.9%) of 225 samples showed positive tear RT-PCR results. Five patients had positive RT-PCR results with all of the methods and 12 patients showed positive results with one of the methods (14). Seah et al. also used Schirmer's test methods while the other authors used conjunctival swab technique (8). Arora et al. claimed that conjunctival swab is the gold standart technique for collecting tear samples.

The different results between these studies can be explained with the differences in sampling time, sampling method, number of samples taken, the expert taken the sample, the patient's compliance as well as the viral load and viral transmission to tears which vary from one patient to another.

We collected nasopharyngeal and tear samples on the first day of admission from all patients. In our study the mean time of the onset of the systemic symptoms by the time of sampling was 2.53±2.21 (1-8) days. The viral load seems to fall in the following weeks of symptoms (20). Arora et al. performed ocular sampling within 48 hours of confirmatory nasopharyngeal swabs. Also Karimi from Iran took samples on the first day of admission when the viral load is thought to be high and found positivity rate of 7% in tear samples. They claimed that racial variation and differences and the variation in virus subtypes in different regions may contribute the positive rates (15). Ziad et al. reported that in tracheal aspirates SARS-CoV-2 concentration was higher than the nasopharyngeal sample (21). This sampling result shows that viral load differs in different parts and secretions. Table-2 shows the results of studies including ocular manifestations, nasopharyngeal and ocular samples of the patients with COVID-19.

Liang et al. estimate the viral load is proportional to the severity of the disease (22). In our study, RT-PCR of nasopharyngeal sample results was positive in nine (60%) of 15 patients. Among these patients four were severe and five were moderate while two of the patients with negatif PCR were severe and four moderate. In the study of Wu et al., 28 of 38 patients had positive results from the nasopharyngeal samples. Among them four had moderate, two had severe and six had critical disease. Tear samples showed positive results in two patients with critical disease (23). In a study from Wuhan, of the 121 patients analysed, three patients had positive results in tears, with two of them classified as severe/critical disease and another classified as mild/moderate (20).

Due to the low virus concentration in non-inflamed structures, ocular involvement is essential for viral shedding in tears. Many studies have indicated variable presentations of COVID-19 patients including ocular manifestations with laboratory reports. Guangfa Wang, a Chinese doctor, first reported that he was infected via ocular secretions. Before onset of pneumonia, he complained of redness of the eyes (24).

Table-1. Demographic features, clinical profiles and laboratory results of the patients with COVID-19.

No	Sex	Age	Systemic symptoms	Ocular symptoms	Severity of the disease/ oxygen saturation	Tear sample results	Nasopharyngeal sample results	Systemic disease
1	F	48	coughing, dyspnea, joint pain, loss of appetite	none	severe spO_2<90	Negative	Positive	one kidney
2	M	62	coughing, dyspnea, fatique	none	severe spO_2<90	Negative	Positive	hypertension
3	F	76	dyspnea, headache, fatique	none	moderate spO2>90	Negative	Negative	hypertension
4	M	32	coughing, dyspnea	none	moderate spO2>90	Negative	Negative	none
5	F	44	coughing	none	moderate spO2>90	Negative	Negative	focal segmental glomerulosclerosis , rheumatoid artritis
6	M	21	coughing, dyspnea,diarrhea	none	severe spO_2<90	Negative	Negative	testicular cancer
7	M	40	fever, joint pain	redness, itching, burring	moderate spO2>90	Negative	Positive	diabetes mellitus, hypertension, trombocytopenia, chronic hepatitis B
8	F	56	coughing	none	moderate spO2>90	Negative	Positive	diabetes mellitus, hypertension, Crohn disease
9	F	84	fatique, loss of appetite	redness, itching, burring	moderate spO2>90	Negative	Positive	Alzheimer disease, chronic heart failure
10	M	46	dyspnea, nausea	none	severe spO_2<90	Negative	Negative	asthma
11	M	77	dyspnea, fever, fatique, loss of appetite	none	severe spO_2<90	Negative	Positive	hypertension, chronic lymphocytic leukemia
12	F	89	dyspnea	none	severe spO_2<90	Negative	Positive	Alzheimer disease, hypertension, cerebrovascular occlusive disease
13	M	57	coughing, fever, headache, fatique	none	moderate spO2>90	Negative	Positive	hypertension
14	M	35	fatique	none	moderate spO2>90	Negative	Positive	leukemia
15	M	41	coughing, fever	none	moderate spO2>90	Negative	Negative	

Table-2. Results of studies including ocular manifestations, nasopharyngeal and ocular samples of the patients with COVID-19.

Publication	Number of patients	Number of nasopharyngeal swabs/positive results	Ocular sampling method	Number of ocular swabs/positive results	Number of patients with positive ocular swabs and positive nasopharyngeal swabs	Number of patients with ocular manifestations	Number of patients with positive ocular swabs and having ocular manifestations	Other features
Kaya et al (13). Turkiye	32	32/16	Conjunctival swab	32/5	3	0	0	
Karimi et al (15). Iran	43	43/30	Conjunctival swab	43/3	3	1	1	
Atum et al (17). Turkiye	40	Not specified	Conjunctival swab	40/3	Not specified	10	1	
Xia et al (11). China	30	They collected sputum sample	Conjunctival swab	60/2	They collected sputum sample	1	1	They collected sputum sample
Zhang et al (12). China	72	72/72	Conjunctival swab	72/1	1	2	1	
Zhou et al (16). China	121	121/121	Conjunctival swab	121/3	3	8	1	
Arora et al (14). India	78	75/75	Conjunctival swab, schirmer's strip	225/29	18	0	0	They collected tear samples via 2 methods and grouped the patients into 3
Kumar et al (19). India	45	45/45	Conjunctival swab	45/1	1	0	0	
Seah et al (8). Singapore	17	17/17	Schirmer's strip	64/0	0	0	0	Samples from both eyes at varying time points
Azzolini et al (10) Italy	91	91/58	Conjunctival swab	91/52	15	NA	NA	
Kocamış et al (18). Turkiye	45	45/45	Conjunctival swab	45/1	1	0	0	

Cheema et al. reported a 29 year old female presenting with keratoconjunctivitis as the main symptom of COVID-19 (25). In a study of 1099 patients with confirmed COVID-19, nine patients (0.8%) had conjunctival manifestations (26). Zhou et al. reported ocular symptoms in eight (6.6%) out of 121 patients including itching, redness, tearing, discharge and foreign body sensation. Of the three (2.5%) patients with positive sample results in tears, one had symtoms while the other two were asymptomatic (20). Three studies from Turkey evaluated conjunctival swab PCR results in patients with COVID-19. Atum et al. collected tears samples from 40 patients with confirmed COVID-19 and three (7.5%) were positive. Ten of the patients had conjunctivitis and of the 10 patients only one had positive conjunctival sample result (17). Other study from Turkey, Kaya et al. evaluated SARS-CoV-2 in tears of 32 patients. Their patients did not have ocular manifestations. Five (16%) patients yielded positive tear PCR results (13). Out of 45 confirmed COVID-19 patients Kocamış et al. reported that conjunctival swab was positive in only one patient (2.22%) and none of the 45 patients had ocular signs and symptoms (18).

RT-PCR have some limitations in its use in ocular samples. Low concentrations of viral RNA in ocular secretions, as well as a possible limited duration of viral shedding may contribute to false negative reports (9). Difficulty in collecting adequate amount of tear sample also contributes to negative results. These studies indicate that SARV-CoV-2 RNA can be shown in very small percantage of COVID-19 patients; however these results don't eliminate the transmission of the virus through ocular tissues and secretions. Collecting a tear sample at the same time with the nasopharyngeal sample may contribute to the diagnosis of the disease by increasing the probability of detecting the virus. In addition physicians must be suspicious that tears of patients may be infective despite the absence of ocular manifestations.

These study have some limitations including small sample size, only one time sampling, absence of detailed ocular examination as well as absence of a control group. Further studies are necessary to evalute the presence and role of novel Coronavirus in COVID-19 disease.

CONCLUSION

Although the results of our study suggest that the risk of SARS-CoV-2 transmission through tears is extremely low; the potential for the SARS-CoV-2 for conjunctival transmission is worth further exploration. The negative tear RT-PCR results should be re-evaluated with multiple samplings done at varied intervals.

Conflict of interest: The authors have no conflict of interest.

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