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ORIGINAL ARTICLE

Evaluation of Nasopharyngeal and Conjunctival Swab Samples of Hospitalised Patients with Confirmed COVID-19

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ABSTRACT

Purpose: To evaluate the results of conjunctival and nasopharyngeal swab tests in patients with confirmed COVID-19.

Methods: This prospective study included 45 patients who were hospitalized for confirmed COVID-19. Nasopharyngeal swab samples were obtained from the patients before hospitalization. Only one eye of each patient was randomly selected for-conjunctival sampling. All participants underwent a complete slit-lamp examination. Conjunctival and nasopharyngeal swab samples were analyzed by reversetranscriptase-polymerase-chain reaction (RT-PCR).

Results: Twenty seven (60%) of the patients were male and 18 (40%) were female. Conjunctival swab was positive in only one (2.22%) patient. None of the COVID-19 patients showed ocular changes and symptoms. There were no abnormalities of the ocular surface, anterior chamber or posterior segment at slit-lamp examination.

Conclusions: The RT-PCR was not high positive in the conjunctiva as in nasopharyngeal swabs. Ocular changes were not common in COVID-19 patients.

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COVID-19; nasopharyngeal; conjunctival; swab; slit lamp

As known, coronavirus disease-19 (COVID-19) first started in Wuhan city of China and spread rapidly all over the world. On January 7, 2020, the China Center for Disease Control isolated this pathogen as a new species. On March 11, 2020, the World Health Organization officially declared the COVID-19 outbreak as a pandemic.[1]

COVID-19 disease is caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), previously called 2019 new coronavirus (2019-nCoV). SARS-CoV-2 is a single-stranded positive sense (sensitive) enveloped RNA virus.[2] The main route of transmission is from person to person through direct contact with droplets; transmission from asymptomatic carriers has also been reported.[3] The signs and symptoms of COVID-19 have been described as fever, cough, shortness of breath, muscle pain, fatigue, sputum production, headache, hemoptysis, diarrhea and conjunctivitis.[4]

There is no specific treatment for COVID-19.The diagnosis is mainly based-on typical symptoms, pulmonary involvement, and exposure to infected patients and is confirmed by positive nucleic acid test of SARS-CoV-2 from various types of samples. Reverse transcriptase-polymerase-chain-reaction (RT-PCR) is the most common method for SARS-CoV-2 detection. Oropharyngeal and nasopharyngeal swabs are the most frequently used samples.

Up to now, limited number of studies have been conducted to understand the transmission of SARS-CoV-2 and the clinical features of the disease on the ocular surface. A recent study has shown that SARS-CoV-2 can be detected in the conjunctival sac of COVID-19 patients.[5]

In this study, we aimed to evaluate the presence of SARS-CoV-2 agent in conjunctival secretion and the possibility of a transmission route by using conjunctival swabs from the patients who were newly diagnosed as COVID-19 using the RT-PCR method.

Methods

This prospective study included 45 patients who were hospitalized for confirmed COVID-19 at the University Hospital. All patients had nasopharyngeal swab positivity with SARS-CoV -2RT-PCR test before hospitalization. Before collecting conjunctival samples, each patient was informed about the aims and methods of the study and informed consent was obtained from all patients. The research was-approved by the Ministry of Health and institutional ethics committee of the University Hospital.

Those with suspect COVID-19 or negative RT-PCR test were not included in the study. All participants underwent a biomicroscopic slit-lamp examination before sampling to rule out the presence of ocular surface infection or any ocular disease. Patients with the diagnoses of on-going ocular or-systemic infection, topical or systemic use of antibiotics before sampling, chronic use of ocular drops as well as those who had any previous ocular surgery were excluded.

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Swab samples were obtained from both eyes and only one eye of each patient was randomly selected for the study. All conjunctival swabs were collected on the same day. The samples were obtained by swabbing the inferior conjunctival fornix and inferior tarsal conjunctiva with a sterile cotton swab without touching the eyelid margins or eyelashes, without application of a topical anesthetic by an experienced ophthalmologist wearing full personal protective equipment.

Specimens were immediately sent to the-COVID-19 laboratory of the university in Bio-Speedy COVID-19 transfer tube (Bioeksen, Turkey) containing optimized viral nucleic acid buffer. The tubes were vortexed for 20 seconds and transferred to a new PCR tube to obtain 100 µl for PCR test. Five microliters were used as template nucleic acid. Extracted samples were studied with SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) gene and the human RNase P gene containing PCR primer probe sets in Bio-Speedy RT-qPCR kit (Bioeksen, Turkey) in accordance with the manufacturer's instructions. The amplification steps were as follows; the protocol was applied for cDNA synthesis, 1 cycle of 15 minutes at 45 °C, for pre-denaturation 1 cycle of 3 min at 95 °C, and for replication and reading step 45 cycles of 5 seconds at 95 ° C and 35 seconds at 55 °C. The resulting amplification curves were evaluated on the Roche LightCycler® 480 (Roche Diagnostics, Germany) device and those under 40 CT (cycle threshold) and sigmoidal were considered positive.

Results

The mean age of the COVID-19 patients in the study was 39.64 ± 22.84 (range 4–87) years. Twenty seven (60%) of the patients were male and 18 (40%) were female.

Patients with a minimum age of 4-years and a maximum age of 87 years were present in the study. Seventeen (37,77%) patients were between 20 and 40 years, 6 (13,33%) patients were between 40 and 60 years, 12 (26,66%) patients were more than 60 years and 10 (22,22%) patients were less than 20 years.

Nasopharyngeal swab test results of all patients were found positive by RT-PCR. The clinical and demographical characteristics of the patients are shown in Table 1. Conjunctival swab test was positive in only one (2.22%) of 45 patients (Table 2). Fifteen (30%) patients had pulmonary changes detected by computerized tomography. In 10 (22.22%) patients, there were abnormal laboratory findings like elevated levels of C-reactive protein and troponine and decreased number of neutrophils.

Adult patients received a treatment combination of favipiravir 800 mg orally twice daily on day 1 and 600 mg orally twice daily on days 2–5, hydroxychloroquine 200 mg twice daily for 5 days and enoxaparin 60 mg daily. Only hydroxychloroquine 1 × 200 mg/day was started to the pediatric patients. Remdesivir 400 mg twice daily was used for 5 days in a pregnant patient who was positive for COVID-19.

None of the patients, both symptomatic/asymptomatic and those with positive chest tomography findings, had coexisting ocular symptoms or ocular changes. There were no abnormalities of the ocular surface, anterior chamber or posterior segment at slit-lamp examination.

Discussion

COVID-19 is diagnosed based on the detection of genetic material from the virus by molecular microbiological methods in a patient sample. Conjunctival swab sampling with PCR test is a noninvasive diagnostic method for identifying the infection of COVID-19. Nasopharyngeal or saliva samples obtained from suspect patients with high fever, shortness of breath and travel history and those who had close contact with infected are the most common tests using specific RT-PCR kits for SARS-CoV-2to detect the RdRp and S gene variable.[6]

RT-PCR is an effective method to detect viral nucleic acid.[7,8] Due to its high precision and specificity, this method has the advantages of simplicity, convenience, and efficiency. Some researchers have found that RT–PCR is effective for the diagnosis of new coronavirus and is better than smear staining examination and culture identification. It has become the gold standard for the diagnosis of coronavirus infections. However, there are still false positive and false negative results that inevitably result from sample contamination, damage to genetic material or insufficient viral load in the nasopharyngeal or conjunctival swabs.[9]

Wu et al. examined 38 COVID-19 patients in their study There were 12 patients with ocular symptoms and 2 patients had positive conjunctival swab test results.[10] In our-study, conjunctival swab was positive in only one patient. In addition, the COVID-19 patients mostly be-longed to-the relatively younger age groups (27 patients were less than 40 years and 33 patients were less than 60 years) and there were no ocular symptoms or changes. Zhou et al.[5] found positive conjunctival swab tests in 3 patients in a series of 67 COVID-19 positive or suspect patients and no patients had reported ocular symptoms. In a single-center observational study, conjunctival swab test was found positive in only one of 72 COVID-19 patients.[11] Xia et al.[12] reported tear and conjunctival swab results of 30 patients examined by PCR and there was only 1 patient with positive conjunctival swab test.

Kumar et al. in a prospective interventional study, evaluated conjunctival swabs concurrent with nasopharyngeal swabs of 45 patients with PCR and found positivity in only 1 patient as in this study. Nasopharyngeal swab test results of all patients were positive with RT-PCR before hospitalization in our study. While Kumar et al. obtained conjunctival swab from only one eye of patients, we had swab samples from both eyes and randomly selected one of them. When two studies are compared in terms of age and gender of patients, we-see that the results are similar. When systemic symptoms are compared, asymptomatic patients were dominant in our study, whereas symptomatic patients were the majority in the study of Kumar et al.[13]

Sindhuja et al.[14] detected ocular findings in 11 of 127 COVID-19 patients with mild symptoms. All patients had positive nasopharyngeal swab test. They found conjunctival congestion in eight of 11 patients.[14] They reported that 3 out of the 8 patients with conjunctivitis had onset of ocular complaints even before the manifestation of COVID-19 symptoms at the time of study.[14] There were no ocular symptoms or signs in our patient group.

In this study, conjunctival swabs of 45 COVID-19 patients were examined by RT-PCR and SARS-CoV-2 RNA was found

Table 1. Clinical and demographical features of COVID-19 patients.

Patient number	Age	Gender	Ocular symptoms	Systemic symptoms	Chest computed tomography	Laboratory
1	21	female	asymptomatic	none	none*	normal
2	31	male	asymptomatic	none	negative	normal
3	16	female	asymptomatic	none	negative	normal
4	62	female	asymptomatic	cough	ground glass opacity	normal
5	9	male	asymptomatic	none	negative	normal
6	39	male	asymptomatic	none	ground glass opacity	normal
7	38	female	asymptomatic	none	negative	normal
8	33	female	asymptomatic	none	ground glass opacity	normal
9	76	male	asymptomatic	none	ground glass opacity	abnormal
10	15	male	asymptomatic	none	negative	normal
11	59	male	asymptomatic	none	negative	abnormal
12	19	female	asymptomatic	none	ground glass opacity	normal
13	4	male	asymptomatic	none	negative	normal
14	56	female	asymptomatic	none	ground glass opacity	abnormal
15	8	male	asymptomatic	none	negative	normal
16	36	female	asymptomatic	none	negative	normal
17	30	female	asymptomatic	none	negative	normal
18	59	male	asymptomatic	shortnessof breath	ground glass opacity	abnormal
19	12	female	asymptomatic	none	negative	normal
20	49	male	asymptomatic	none	negative	normal
21	17	male	asymptomatic	none	negative	normal
22	28	female	asymptomatic	none	negative	normal
23	35	male	asymptomatic	none	negative	normal
24	12	female	asymptomatic	none	negative	normal
25	36	female	asymptomatic	none	negative	normal
26	32	female	asymptomatic	none	negative	normal
27	49	male	asymptomatic	none	negative	normal
28	19	male	asymptomatic	none	negative	normal
29	72	male	asymptomatic	none	ground glass opacity	abnormal
30	64	female	asymptomatic	none	ground glass opacity	normal
31	30	male	asymptomatic	none	ground glass opacity	abnormal
32	62	male	asymptomatic	none	negative	normal
33	61	male	asymptomatic	none	negative	normal
34	24	male	asymptomatic	none	negative	normal
35	62	male	asymptomatic	none	negative	normal
36	64	female	asymptomatic	none	negative	normal
37	80	male	asymptomatic	none	ground glass opacity	abnormal
38	25	male	asymptomatic	none	negative	normal
39	38	male	asymptomatic	none	negative	normal
40	32	male	asymptomatic	none	ground glass opacity	normal
41	28	male	asymptomatic	none	negative	normal
42	73	male	asymptomatic	chest pain	ground glass opacity	abnormal
43	86	female	asymptomatic	shortnessof breath	ground glass opacity	abnormal
44	87	male	asymptomatic	shortnessof breath	ground glass opacity	abnormal
45	46	female	asymptomatic	none	negative	normal

*: Pregnant patient.

positive in only 1 (2.23%) patient. In previous studies including this study, lower detection rate of SARS-CoV-2 RNA in conjunctival swabs and lower levels of viral reservoir might have resulted from several factors, such as possible duration for maximum replication of the virus, time of the sampling, patient admission time to the hospital and possibly smaller amount of the virus in conjunctival secretions, as well as decreased sensitivity of RT-PCR test.[15]The results have revealed that nasopharyngeal swabs showed much more positive rates than conjunctival swabs for SARS-CoV-2 detection and conjunctival swabs may result in a high negative rate.

The only positive conjunctival swab test was detected in a pregnant patient. There are limited data about treatment options for pregnant women with COVID-19. As the pandemic continues and pregnant women remain at risk effective drugs such as remdesivir is crucial for this patient group.[16] Remdesivir is a nucleoside analogues drug with extensive antiviral activity. On the other hand, pregnancy is a period of partial immune suppression that may make pregnant women more vulnerable to viral infections. Therefore, we may propose that the conjunctival swab positivity may be attributed to the presence of pregnancy instead of treatment protocol.

Number of male patients were more than females in our study. Sixty percent of the patients were male and 40% were female. The reduced susceptibility of females to COVID-19 was observed in previous results.[8] In the study, the gender difference in COVID-19 could not be shown due to the relatively lower number of patients included and because the study was conducted for the nasopharyngeal and conjunctival swab test results and not to detect gender differences in the COVID-19 disease.

Our study had some limitations. First, the number of patients was relatively small. Second, conjunctival swab sampling was done only once for each patient. This might have reduced the possibility

Table 2. Conjunctival and nasopharyngeal swab test results of COVID-19 patients.

Patient number	Time of conjunctivalswab (days)	Conjunctival swab	Nasopharyngeal swab
1	3	positive	positive
2	4	negative	positive
3	5	negative	positive
4	4	negative	positive
5	2	negative	positive
6	6	negative	positive
7	5	negative	positive
8	5	negative	positive
9	4	negative	positive
10	3	negative	positive
11	3	negative	positive
12	5	negative	positive
13	5	negative	positive
14	8	negative	positive
15	5	negative	positive
16	5	negative	positive
17	3	negative	positive
18	7	negative	positive
19	5	negative	positive
20	5	negative	positive
21	5	negative	positive
22	3	negative	positive
23	3	negative	positive
24	6	negative	positive
25	6	negative	positive
26	3	negative	positive
27	4	negative	positive
28	3	negative	positive
29	3	negative	positive
30	3	negative	positive
31	3	negative	positive
32	2	negative	positive
33	4	negative	positive
34	4	negative	positive
35	4	negative	positive
36	3	negative	positive
37	3	negative	positive
38	5	negative	positive
39	5	negative	positive
40	3	negative	positive
41	4	negative	positive
42	6	negative	positive
43	3	negative	positive
44	4	negative	positive
45	3	negative	positive

of isolating the virus. On the other hand, by using full personal protective equipments, two of the authors, who were also among the opthalmic consultants of COVID-19 service, performed slit-lamp examinations to rule out the presence of any type of ocular surface infection or any occult ocular changes. All patients were under treatment during the study and there were no outpatient COVID-19 cases in our study. Those with suspicious COVID-19 or negative RT-PCR test were not included in the study. These were the strengths of the study.

To conclude, our study suggests that nasopharyngeal swabs are still the most valuable sampling type-in comparison to conjunctival swabs-during COVID-19 outbreak. Since ocular changes are not common both-in symptomatic and-asymptomatic patients, ophthalmological examination may-not be included as part of routine evaluation in COVID-19 patients. In this study, we have-shown that SARS-CoV-2 virus may be found in the conjunctival swabs of hospitalized COVID 19 patients. Despite the-lower rate of detection, necessary precautions should always be taken during opthalmological examination.

Disclosure statement

None of authors has conflict of interest with this submission.

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