Original Article

Presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of COVID-19 patients

Kiran Kumar, Akshata A Prakash, Suresh Babu Gangasagara, Sujatha Rathod BL, K Ravi[1], Ambica Rangaiah[2], Sathyanarayan Muthur Shankar[2], Shantala Gowdara Basawarajappa[2], Shashi Bhushan[3], T G Neeraja, Srinivas Khandenahalli, M Swetha, Priyam Gupta, U C Sampritha, Guru N S Prasad, Chakravarthy Raghunathan Jayanthi[4]

Purpose: To detect the presence of viral RNA of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in conjunctival swab specimens of coronavirus disease-19 (COVID-19) patients. **Methods:** Forty-five COVID-19 patients positive for real-time reverse transcription-polymerase chain reaction (RT-PCR) for SARS-CoV-2 in nasopharyngeal swab with or without ocular manifestations were included in the study. The conjunctival swab of each patient was collected by an ophthalmologist posted for COVID duty. **Results:** Out of 45 patients, 35 (77.77%) were males and the rest were females. The mean age was 31.26 ± 12.81 years. None of the patients had any ocular manifestations. One (2.23%) out of 45 patients was positive for RT-PCR SARS-CoV-2 in the conjunctival swab. **Conclusion:** This study shows that SARS-CoV-2 can be detected in conjunctival swabs of confirmed cases of COVID-19 patients. Though the positivity rate of detecting SARS-CoV-2 in conjunctival swabs is very less, care should be exercised during the ocular examination of patients of COVID-19.

Key words: Conjunctival swab, coronavirus disease-19, nasopharyngeal swab, SARS-CoV-2

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Since December 2019, there have been cases of coronavirus disease 2019 (COVID-19) reported in China, which soon spread to other parts of the world. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative pathogen which is a member of the Coronaviridae family. A few researchers have reported keratoconjunctivitis as an ocular abnormality of SARS-CoV-2.[1] Signs and symptoms of COVID-19 have been described as fever, cough, myalgia, fatigue, sputum production, headache, hemoptysis, diarrhea, and conjunctivitis.[2] Since the diagnosis of COVID-19 patients cannot be only based on symptoms, reverse transcription-polymerase chain reaction (RT-PCR) is a simple and reliable molecular test on respiratory samples (throat swab/nasopharyngeal swab/sputum/endotracheal aspirates, and bronchoalveolar lavage).[3]

Close examination of patients by ophthalmologists and physical contact with patients' eyes is inevitable. This could be a potential source of the spread of SARS-CoV-2. Other viruses like adenovirus and influenza viruses are known for ocular tropism.[4] Therefore, this study was designed to understand whether SARS-CoV-2 can be detected in the conjunctival swab of patients of COVID-19.

Methods

A prospective interventional study was conducted at a dedicated tertiary COVID-19 hospital in south India in April 2020. Institute ethical clearance was obtained prior to the onset of the study. Confirmed positive cases of COVID-19 by nasopharyngeal swab according to WHO standards with or without ocular symptoms were included in the study.[5] Suspect and probable cases and those patients who were critically ill were excluded. Signed consent was obtained for each sample collection from patients. The conjunctival swab was collected by an ophthalmologist posted for COVID duty. Eyelids were everted and samples were obtained by sweeping the inferior fornices of either of the two eyes with sterile nylon flocked swabs without topical anesthesia. The tips of the swab sticks were broken off and placed into a viral transport medium—Hi media (HiMedia Laboratories Pvt. Ltd, Nashik, India. Catalog No: 0000431084). Personal protection equipment was used and necessary precautions were used to minimize the risk of spread of infection from one patient to another. Samples were transported to the specified lab facility maintaining the recommended cold chain. In the laboratory,

Department of Ophthalmology, Minto Ophthalmic Hospital, Bangalore Medical College and Research Institute, Departments of [1]Medicine, [2]Microbiology, [3]Pulmonary Medicine and [4]Department of Pharmacology, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India

Correspondence to: Dr. Kiran Kumar K, Cataract, Cornea and Refractive Services, Minto Regional Institute of Ophthalmology, Bangalore Medical College and Research Institute, Bengaluru - 560 002, Karnataka, India. E-mail: dockiran2011@gmail.com

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Table 1: Demography, clinical symptoms, nasopharyngeal swab, and conjunctival swab reports of coronavirus disease-19-positive patients

S. No	Age in years	Sex	History of contacts	Clinical symptoms	Nasopharyngeal swab	Day of conjunctival swab collection after the onset of symptoms	Conjunctival swab
1	32	F	No	Fever, cough	Positive	3	Negative
2	13	M	Yes	Fever, sore throat	Positive	3	Negative
3	25	F	Yes	Asymptomatic	Positive	NA	Negative
4	6	M	Yes	Fever, sore throat, cough	Positive	4	Negative
5	32	M	No	Asymptomatic	Positive	NA	Negative
6	23	M	No	Asymptomatic	Positive	NA	Negative
7	28	M	No	Fever, cough	Positive	2	Negative
8	55	M	Yes	Cough, breathlessness	Positive	4	Negative
9	11	M	No	Fever	Positive	5	Negative
10	21	M	Yes	Asymptomatic	Positive	NA	Negative
11	21	F	Yes	Fever, cough	Positive	3	Negative
12	50	F	Yes	Fever, breathlessness	Positive	4	Negative
13	30	F	Yes	Asymptomatic	Positive	NA	Negative
14	43	M	Yes	Cough, sore throat	Positive	4	Negative
15	22	M	Yes	Cough	Positive	4	Negative
16	40	M	Yes	Fever, cough	Positive	4	Negative
17	24	M	Yes	Asymptomatic	Positive	NA	Negative
18	25	M	Yes	Cough, breathlessness	Positive	3	Negative
19	30	M	Yes	Fever, breathlessness	Positive	2	Negative
20	37	M	Yes	Cough, breathlessness	Positive	5	Negative
21	30	M	Yes	Fever, cough	Positive	4	Negative
22	30	M	Yes	Asymptomatic	Positive	5	Negative
23	41	M	Yes	Fever, breathlessness	Positive	3	Negative
24	49	F	Yes	Cough, breathlessness	Positive	5	Negative
25	31	M	Yes	Fever	Positive	2	Negative
26	26	M	Yes	Asymptomatic	Positive	NA	Negative
27	35	M	Yes	Fever	Positive	4	Negative
28	52	M	Yes	Fever, cough	Positive	3	Negative
29	24	M	Yes	Asymptomatic	Positive	NA	Positive
30	22	M	Yes	Fever, cough	Positive	2	Negative
31	17	M	Yes	Cough	Positive	4	Negative
32	19	F	Yes	Fever	Positive	5	Negative
33	26	M	Yes	Asymptomatic	Positive	NA	Negative
34	58	M	Yes	Fever, breathlessness	Positive	4	Negative
35	28	M	Yes	Cough	Positive	4	Negative
36	21	M	Yes	Asymptomatic	Positive	NA	Negative
37	44	M	Yes	Fever, cough	Positive	4	Negative
38	38	M	Yes	Asymptomatic	Positive	NA	Negative
39	20	M	Yes	Asymptomatic	Positive	NA	Negative
40	34	M	Yes	Fever, cough	Positive	3	Negative
41	27	M	Yes	Fever, cough	Positive	2	Negative
42	66	F	Yes	Fever, cough breathlessness	Positive	4	Negative
43	27	F	Yes	Asymptomatic	Positive	NA	Negative
44	52	F	Yes	Fever, cough	Positive	2	Negative
45	22	M	Yes	Cough, breathlessness	Positive	4	Negative

M=Male; F=Female, NA=Not applicable

samples were extracted using the magnetic bead extraction method (Thermo scientific viral isolation kit—5X MagMAX™, Thermo Fischer scientific Baltics UAB, Vilnius, Lithuania) in Biomek 4000 platform as per the manufacturer's instructions. In eight-strip PCR tubes, 22.5 μL of the mixed reaction solution or master mix (SARS CoV-2 Detection kit; fortitude 2.0) was

loaded. To this, 2.5 µL of the extracted nucleic acid was added to each well, covered and the PCR was performed using the CFX96 real-time PCR (Bio-Rad). After the completion of the PCR run, the amplification curves were judged to decide if the results were negative or positive. A cutoff cycle threshold (Ct) value of 40 was considered as positive as per the manufacturer's instructions.

Results

Out of 45 patients, 35 (77.77%) were males and the rest were females. Patients with a minimum age of 6 years and a maximum age of 75 years were present in the study. For our convenience, patients were divided into four age groups: less than 20 years (6); 21–40 years (29); 41–60 years (9), and more than 60 years (1). The mean age (±SD) was 31.26 ± 12.81 years. None of the patients in our study had any ocular symptoms.

Only one (2.23%) out of 45 patients was positive with the Ct value of 33, for real-time RT-PCR SARS-CoV-2 in conjunctival swab as shown in Table 1.

Discussion

No reports were suggesting ocular transmission of COVID-19 initially. Xia *et al*. evaluated the conjunctival secretions of 30 confirmed cases of COVID-19.[6] In one of these patients, both tear and conjunctival secretions tested positive for the virus by RT-PCR. There are numerous anecdotal reports suggesting conjunctivitis as the initial symptom before the onset of pneumonia, including that of Guangfa Wang, a national expert on the panel for pneumonia during the early investigations in Wuhan, China.[7]

Some studies reported the presence of SARS-CoV or Middle-East respiratory syndrome (MERS-CoV) in tears or conjunctival sac.[8] Another study by Chen *et al*. detected SARS-CoV-2 in the conjunctival sac of only three COVID-19 patients out of 67 positive cases. But these three patients did not have any ocular symptoms.[9] Sun *et al*. found that among 72 patients confirmed by laboratory diagnosis with SARS-CoV-2 RT-PCR assay, SARS-CoV-2 RNA fragments were found in ocular discharges belonging to one patient.[10] The above studies show that SARS-CoV-2 can be detected in conjunctival sac, in only a small percentage of COVID-19 positive patients. Also, the risk of infection of SARS-CoV-2 through eye secretions remains uncertain.

Our study showed that only one (2.23%) out of 45 patients had detectable levels of SARS-CoV-2 in conjunctival swabs of COVID-19-positive patients using RT-PCR. Low level of viral detection can be due to various factors like the time for maximum replication of the virus, the timing of performing of sampling, time of presentation of the patient to the hospital and possibly less secretion of the virus through conjunctival secretion, and also low sensitivity of RT-PCR.[11] In a study done by Ziad and colleagues, tracheal aspirates yielded a higher SARS-CoV-2 load than a nasopharyngeal swab.[12] This suggests that viral concentration differs in different sites and secretions. A study was done by de Wit *et al.* in the rhesus macaque model with MERS-CoV, which is another type of coronavirus.[13] They found out that MERS-CoV RNA could be detected in the conjunctiva well within 6 days of infection beyond which, it failed to detect the virus. Since the majority of people infected with the SARS-CoV-2 virus are asymptomatic, it is difficult to determine the peak of virus load unless multiple samplings are done at varied intervals.

There are several limitations to this study which include relatively small sample size, absence of detailed ocular examinations, sampling done only once from the eyes of each patient, which can increase the prevalence of false-negatives.

Conclusion

This study shows that SARS-CoV-2 can be detected in conjunctival swabs of confirmed cases of COVID-19 patients. Though the positivity rate of detecting SARS-CoV-2 in conjunctival swabs is very less, care should be exercised during the ocular examination of patients of COVID-19.

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Conflicts of interest

There are no conflicts of interest.

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