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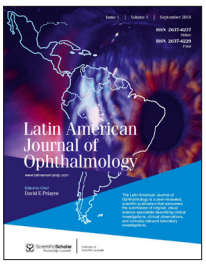

Original Article

Estimation of conjunctival swab and nasopharyngeal swab specimens for viral nucleic acid detection in Coronavirus disease 2019 patients to compare the viral load

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Received : 24 January 2021
Accepted : 26 February 2021
Published : 31 March 2021

DOI
10.25259/LAJO_1_2021

Quick Response Code:

ABSTRACT

Objectives: The purpose of the study was to detect the presence of viral ribonucleic acid of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in conjunctival swab along with nasopharyngeal swab specimens of Coronavirus disease 2019 (COVID-19) patients.

Material and Methods: Thirty COVID-19 patients with at least one sample positive for real-time reverse transcription-polymerase chain reaction for SARS-CoV-2 in nasopharyngeal swab with the presence or absence of ocular manifestations were included in the study. The conjunctival swab along with nasopharyngeal swab of each patient was collected and sent to microbiology lab for evaluation and analysis of viral nucleic acid to assess the viral load.

Results: Out of 30 patients, 21 patients (70%) were males and the remaining nine patients (30%) were females. Mean age of the patients in the study was 44.80 ± 15.37 years. One patient had conjunctivitis as ocular manifestation. Two (6.7%) out of 30 patients were positive for RT-PCR SARS-CoV-2 in the conjunctival swab. There was no statistical correlation between nasopharyngeal swab and conjunctival swab positivity using Pearson's correlation coefficient (r) = 0.010; $P = 0.995$ (>0.05).

Conclusion: The results of the study revealed that SARS-CoV-2 can also be detected in conjunctival swabs of confirmed cases of COVID-19 patients. Although, in comparison to nasopharyngeal and throat swabs the rate of detection of SARS-CoV-2 in conjunctival swabs is relatively less, still diligent care and precautions should be practiced during the ophthalmic evaluation of COVID-19 patients.

Keywords: Conjunctival swab, Nasopharyngeal swab, Severe acute respiratory syndrome coronavirus-2, Reverse transcription polymerase chain reaction

INTRODUCTION

Coronavirus disease 2019 (COVID-19); the ongoing global major health hazard is caused by the highly transmissible novel severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) which originated in China in December 2019 and rapidly became a global pandemic posing serious health threat, recognized by the World Health Organization, with the imminent potential of taking a toll with accelerated overburdening of the health-care systems and causing substantial

mortality worldwide.[1] The vicious cycle of close contact human transmission occurs mainly through respiratory droplets, but other routes are under investigation, because SARS-CoV-2 has also been detected in several other body fluids.[2]

The COVID-19 patients mostly present with signs and symptoms of fever, cough, myalgia, fatigue, sputum production, headache, hemoptysis, diarrhea, and conjunctivitis[3] Although, the fact that the clinical presentation can lead to a provisional diagnosis, but the confirmatory diagnosis of COVID-19 patients can be made after confirmation with reverse transcription-polymerase chain reaction (RT-PCR) which is a simple and reliable molecular test done on respiratory samples (throat swab/nasopharyngeal swab/sputum/endotracheal aspirates, and bronchoalveolar lavage).[4]

The respiratory viruses such as adenovirus, respiratory syncytial virus, influenzae virus, and rhino virus are a well-known entity for ocular tropism[5] The conjunctiva may be a potential site of direct viral inoculation as a result of SARS-CoV droplet from the close contact of an infected patients through aerosol route or migration from upper respiratory tract or through hematogenous spread.[6-8]

So far, only limited data are available on ocular sampling from patients with COVID-19. Earlier, during the SARS epidemic, the exposure of ocular surface to infectious fluids was associated with an increased risk for SARS-CoV transmission to healthcare workers prompting a grave concern that respiratory illness could be transmitted through ocular secretions.[6,9]

Similar concerns have been raised with SARS-CoV-2, especially among eye care providers and those on the front lines triaging what could be initial symptoms of COVID-19.

During the ongoing pandemic surge, SARS-CoV-2 ribonucleic acid (RNA) was detected in conjunctival secretions collected from the patient who presented with conjunctivitis from a hospital in China.[10] However, there is a requirement of further studies to evaluate the infectious potential of the SARSCoV-2 RNA detected in the ocular specimens in comparison to standard nasopharyngeal swab specimen and to determine whether transmission may occur through ocular secretions. The aim of the present study is to estimate the conjunctival swab along with nasopharyngeal swab specimens for viral nucleic acid detection in cases of COVID-19 Patients.

MATERIAL AND METHODS

Study design and participants

A prospective interventional study was conducted at a tertiary care COVID-19 hospital in western part of India from June 2020 to July 2020. The location has currently seen a surge of novel coronavirus (nCoV) activity with maximum reported COVID cases. Institutional based ethical clearance was obtained before commencing the study. The inclusion criteria included confirmed positive cases of COVID-19 by at least one nasopharyngeal swab in adherence to WHO standards with or without ocular symptoms. All other patients suffering from non-COVID-19 respiratory ailments, those who were critically ill were excluded from this study. Detailed history of presenting symptoms, contact history, or travel history to endemic areas prone to COVID infection was taken. Patients were evaluated and the baseline clinical signs including temperature, oxygen saturation (SpO2), respiratory rate, and pulse rate were documented at the time of hospitalization. Written, informed, and signed consent was obtained from patients and relatives for each sample collection.

The objective of the current study is to estimate the viral nucleic acid in conjunctival swab and compare the positive rates of 2019-nCoV between conjunctival swabs and nasopharyngeal swab specimens for the detection of viral nucleic acid by RT-PCR to improve the diagnostic efficiency of COVID-19 and to prevent the onward infection and transmission of the disease entity through ocular route.

Specimen collection

The conjunctival swab was collected along with the other nasopharyngeal swab under strict aseptic conditions. Personal protection equipment was donned at all times while collecting the samples and adequate precautions were taken to decimate the possibility of contamination of sample or risk. The specimen was collected by sweeping the inferior fornices of either of the two eyes with a sterile nylon flocked swab stick without instillation of topical anesthesia after gently everting the eyelids. Subsequently, the tips of the swab sticks were broken off after collecting the sample and placed into a viral transport medium (HiMedia Laboratories Pvt. Ltd, Nashik, India) and were transferred to the microbiology department of this institute without any interruption to the cold chain for further analysis where real-time RT-PCR was performed to detect the viral RNA genome and virus load in each samples of spread of infection from one patient to another. Real-time RT-PCR was carried out using commercial, Indian Council of Medical Research approved SARS-CoV 2 RT-PCR kit [Qiagen™(Qiagen N. V., Netherlands).

RT PCR protocol

In the laboratory, the samples were extracted in Biomek 4000 platform as per the manufacturer's instructions. RNA extraction of clinical samples was performed according to "RNA extraction-QIAmp viral RNA Mini Kit" protocol in

RNA extraction area. RT-PCR were run including negative control, positive control, and MOCK (human source cell line) in the test. Centrifugation was done for 5–10 s to collect contents at bottom of the tube. Reaction strip tubes or plates were setup in 96-well cooler rack. 20 µl of each master mix was dispensed into each well as per the plate set up. In the nucleic acid extraction room, 5 µl of each sample and 5 µl of mock extraction control were added into respective wells as per the set up. Finally, 5 µl of positive viral template control was pipetted into all viral template control wells. After the completion of the PCR run, the amplification curves were used to decide the negative or positive result. A cutoff cycle threshold (Ct) value of <35 was considered as positive as per the manufacturer's instructions.

Statistical analysis

The statistical analysis was performed using SPSS 22.0. Continuous variables were described using mean (SD), if they are normally distributed. Categorical variables were expressed as frequencies (percentages). Fisher extract test was performed to find out the significant correlations. The value of $P < 0.05$ was considered as statistically significant. Correlation plot between nasopharyngeal swab and conjunctival swab was derived using Pearson's correlation coefficient.

RESULTS

Total 30 confirmed positive cases of COVID-19 by at least one nasopharyngeal swab in adherence to the WHO standards with or without ocular symptoms were included in the study. Out of 30 patients, 21 patients (70%) were males and the remaining nine patients (30%) were females. Mean age of the patients in the study was 44.80 ± 15.37 years with age ranging between 18 years and 80 years. Mostly, the patients were in the age group of 41–60 years [Table 1]. The mean time of first collection of tear and conjunctival secretions, from the onset of symptoms was 2.30 ± 0.79 days. Twelve patients (40%) had a history of contact to COVID positive patients whereas six patients (20%) gave history of travel to endemic areas. There was no correlation between exposure and travel history with conjunctival swab positivity ($P > 0.05$ using Chi-square test).

The patients presented commonly with the symptoms of fever, sore throat, cough, and breathlessness, as shown in Table 2. There was an overlap of symptoms in certain patients. However, the correlation between clinical symptoms and conjunctival swab positivity was not statistically significant ($P > 0.05$ using Fisher's exact test) [Table 3].

Only one patient (3.3%) in our study was diagnosed with acute conjunctivitis as ocular manifestation after 1 week of hospitalization. Two patients (6.7%) out of 30 patients were positive for real-time RT-PCR SARS-CoV-2 in conjunctival swab with the Ct value of <35. However, three patients had weakly positive Ct value and the result was inconclusive which on repeat testing came out to be negative. There was no statistically significant correlation between nasopharyngeal and conjunctival swab positivity using Pearson's correlation coefficient (r) = 0.010; $P = 0.995$ (> 0.05) [Graph 1]. The mean body temperature of the patients was 35.81 ± 1.32°C and average SpO2 was 95.63 ± 2.82% at the time of sampling. The statistical correlation between oxygen saturation level of the patient at the time of admission and conjunctival swab positivity ($P = 0.418$ using Fisher's extract test) [Graph 2].

Table 1: Distribution of the patients as per the age group in the study population.

Age group (years)	Number of patients	Percentage
≤20	1	3.3
21–40	12	40.0
41–60	13	43.3
>60	4	13.3
Total	30	100.0

Table 2: Symptomatology of the patients in the study population.

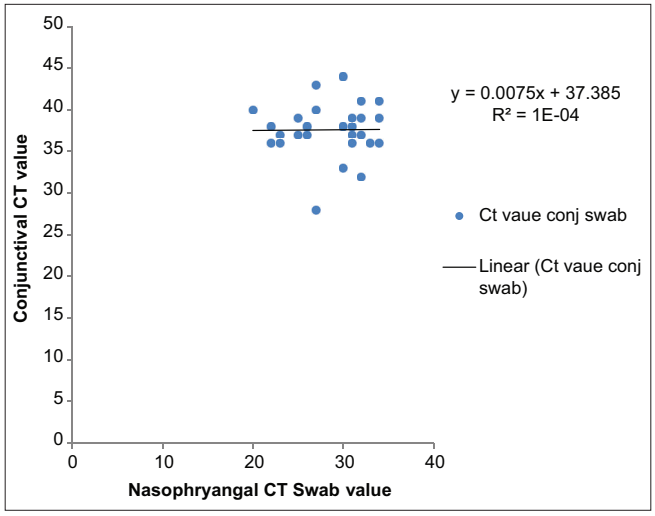
Symptoms	Number of patients	Percentage
Fever	19	63.3
Sore Throat	15	50.0
Breathlessness	9	30.0
Nausea	2	6.7
Vomiting	2	6.7
Cough	11	36.7
Dry Cough	3	10.0

Table 3: Statistical correlation between clinical symptoms and conjunctival swab positivity.

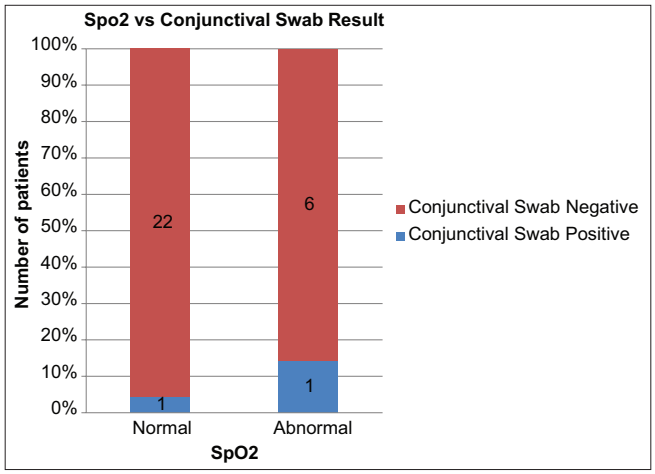
Clinical Symptoms	Conjunctival Swab Results		Total	
	Positive	Negative		
Fever	2	17	19	0.519
Sore throat	1	14	15	0.999
Breathlessness	2	7	9	0.082
Nausea	0	2	2	0.999
Vomiting	0	2	2	0.999
Cough	0	11	11	0.519
Dry cough	0	3	3	0.999
Ocular manifestations	0	1	1	0.999

DISCUSSION

Of all the confirmed COVID-19 cases enrolled in our study, viral nucleic acid was detected in the conjunctival secretions of two patients (6.7%). Only one patient reported with the



Graph 1: Correlation between Ct values of nasopharyngeal swab and Conjunctival swab.



Graph 2: Correlation between oxygen saturation and conjunctival swab positivity.

ocular symptoms of redness and discharge from both the eyes and was diagnosed as Acute Conjunctivitis on the 7th day of admission. The demonstration of the direct association between conjunctivitis and SARS-CoV-2 infection is a diagnostic challenge. In our study, conjunctivitis was seen in conjunctival swab RT-PCR negative patient.[11] However, based on previous results, the extremely low positive rate of SARS-CoV-2 RNA test by RT-PCR in tears and conjunctival secretions from patients with laboratory confirmed SARS-CoV-2 implies that negative test results could be false negative, not excluding the presence of the virus.

During the emergence of the disease, ocular transmission of COVID-19 was not reported initially. However, recent studies have thrown light that SARS-CoV-2 can be detected in conjunctival sac, seen only in smaller percentage of COVID-19 positive patients. Earlier, during the ongoing SARS-CoV infection, studies had reported the presence of virus in the tear and conjunctival fluid[6,9,12] At present, limited data are available on ocular sampling from patients with COVID-19.

Xia *et al.* reported one case who tested positive for the virus by RT-PCR in both tear and conjunctival secretions, after evaluating the conjunctival secretions of 30 confirmed cases of COVID-19.[13]

Study done by Chen *et al.* also reported the presence of SARS-CoV-2 in the conjunctival sac of three patients out of 67 COVID-19 positive cases confirmed by laboratory diagnosis with SARS-CoV-2 RT-PCR assay.[14]

This is a prospective interventional study on a small sample size of 30 confirmed COVID-19 patients. We collected their conjunctival swab samples which were subsequently subjected to RT-PCR Assay. Virus detection in conjunctival swab was seen in only two patients possibly due to factors such as sampling time and amount, peak replication time of the virus, time of presentation of the patient to the hospital, and possibly low sensitivity of RT-PCR.[15]

The current trend of the infected patients with the ongoing SARS-CoV-2 virus shows that mostly the patients are asymptomatic; hence, it is difficult to evaluate the peak virus load in such patients unless multiple sampling at different time frame has been done.

Furthermore, the conjunctival swab and tear film sampling of the convalescent patients should be done to assess the presence and infectivity of the 2019-nCoV in the tear film of such patients.

Although, the current study shows a low risk of coronavirus transmission through the ocular secretion, the need of eye protection in the form of protective goggles, face shields to avoid the eye exposure to disease cannot be overlooked. Especially, the ophthalmologists due to their close proximity to the patients are vulnerable to the risk of acquiring the infections. Hence, safety measures in the form of face shields, slit lamp shields, hand gloves, and protective eye goggles should be encouraged in the routine practice.[16] This study endeavors to provide insights on the feasibility of using ocular tissue or even tears as a medium of diagnosis.

There were certain unavoidable limitations of this study including a smaller sample size, cursory ocular examination, and chances of increased false-negatives due to single time sampling done.

CONCLUSION

The present study successfully concluded that SARS-CoV-2 can also be detected in conjunctival swabs of the COVID-19 patients. However, definitive studies are required in future to assess the exact infectivity rate. Recently, it has been proposed that angiotensin converting enzyme 2 receptors

play a key role in infecting the cells and facilitate in horizontal transmission of the disease. More extensive studies are required in future to prove this hypothesis. Finally, future studies should consider the association between serum viral load and viral shedding in tears. Although the fact, that the rate of detection of SARS-CoV-2 in conjunctival swabs is less, in comparison to standard nasopharyngeal swabs, diligent care and precaution should be practiced while doing the ocular examination of patients of COVID-19 as close contact with the patients by the ophthalmologists is inevitable.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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How to cite this article: Kaushik J, Marwah V, Singh A, Chaitanya YV, Gupta RM, Kumar P. Estimation of conjunctival swab and nasopharyngeal swab specimens for viral nucleic acid detection in Coronavirus disease 2019 patients to compare the viral load. Lat Am J Ophthalmol 2021;4:2.