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Detection of SARS-CoV-2 RNA in a conjunctival swab sample in real-time-polymerase chain reaction positive COVID-19 patients and its association with comorbidity and severity at a designated COVID-19 hospital in Central India

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Purpose: To evaluate the presence of SARS-CoV-2 RNA in the conjunctival swab sample of positive confirmed COVID-19 patients and to find out its association with comorbidity and severity of COVID-19 disease. **Methods:** We conducted an observational cross-sectional study at a dedicated tertiary COVID-19 hospital in central India for a period of 8 weeks from February 2021to March 2021. We included patients who tested positive for SARS-CoV-2 RNA through nasopharyngeal swab and were above 18 years of age**.** Swab samples have been collected within 48 h of admission. Conjunctival swab was taken from the lower fornix of both eyes and sent to microbiology laboratory for real-time- polymerase chain reaction (RT-PCR). **Results:** Out of 150 patients, conjunctival swab RT-PCR was positive in five patients (3.33%). Two patients had conjunctival manifestations in the form of conjunctivitis but conjunctival swab RT-PCR was negative in those patients. Among the RT-PCR positive patients, two (40%) were from mild, one (20%) was from moderate, and two (40%) were from severe category. No association could be established between conjunctival swab RT-PCR positivity and severity of the disease or associated comorbidity. **Conclusion:** Our study provides evidence that SARS-CoV-2 RNA could be detected in conjunctival secretions, and though the risk is relatively low, the eye may act as source of transmission. Extra caution should be taken by healthcare workers, and use of proper precautions like face shields and goggles should be encouraged.

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The presenting symptoms of SARS-CoV-2 are most commonly found to be fever, dry cough, tiredness, and less commonly body pains, sore throat, diarrhea, headache, loss of taste or smell, and conjunctivitis.[1,2] The mean incubation period is around 5 days.[2,3] with a range of up to 14 days. A positive real-time- polymerase chain reaction (RT-PCR) test in nasopharyngeal swab, for detection of SARS-CoV-2 RNA, is considered to be diagnostic for COVID-19 infection.[1] SARS-CoV-2 enters host cells by binding to the angiotensin-converting enzyme 2 receptor, most commonly found in respiratory and cardiovascular systems. The primary mode of transmission is through respiratory droplets and aerosols, which is well recognized[4,5]; however, ACE 2 receptors are also expressed on the conjunctival epithelial cell surface,[6] making it a potential portal of entry. Despite the above reasoning, in studies conducted till now, the rate of positivity in ocular secretions has been found to vary between 0 and 28%, with higher rates seen in patients with severe COVID-19 disease.[7] According to a recent study, there is a significant possibility of transmission of disease through ocular secretions, despite the low levels of detection of virus.[7]

Therefore, we believe that it is important to determine whether SARS-CoV-2 is capable of transmitting via a similar mechanism. The findings of the study could be of significance, especially for frontline health workers, who can then take necessary steps to protect themselves, as well as prevent the spread of the disease.

Methods

We conducted an observational cross-sectional study at a dedicated tertiary COVID-19 hospital in central India from February 1, 2021 to March 15, 2021. We included patients who tested positive for SARS-CoV-2 through nasopharyngeal swab and were above 18 years of age. Conjunctival swabs were collected within 48 h of admission. Institutional ethical

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clearance was obtained before starting the study. Written informed consent was obtained from all the patients in accordance with Declaration of Helsinki. Patients who were critically ill (on ventilator) were excluded from our study.

The computed tomography (CT) scan of chest was evaluated for the presence and extent of COVID-19 disease, based on the findings of ground glass opacities, crazy-paving pattern, and consolidation. Based on anatomical structure, lungs were divided radiologically into five zones: Right upper lobe, right middle lobe, right lower lobe, left upper lobe, and left lower lobe. On the basis of severity of involvement, each lung lobe was assigned a score: Score 0, 0% involvement; score 1, less than 5% involvement; score 2, 5% to less than 25% involvement; score 3, 25% to less than 50% involvement; score 4, 50% to less than 75% involvement; and score 5, 75% or greater involvement. Scores of individual lobes were added to give the final CT score for both lungs (maximum CT score for both lungs was 25).

Based on the above CT scan scoring system, patients were classified as mild (CT score 1–7), moderate (CT score 8–17), and severe (CT score more than 17).[8-10]

History was obtained from all the patients, and bedside ocular examination was performed with the help of torchlight. The conjunctival swab was collected by a trained ophthalmologist wearing a PPE kit and taking proper necessary precautions. While collecting the conjunctival swab sample, lower eyelid was everted and disposable swab stick swiped through the lower fornix of both the eyes, without using topical anesthesia.

After taking sample from both the eyes, nylon flocked swab stick was placed in viral transport media (VTM) and sent to microbiology department in cold box (temperature 4°C).

After receipt in lab VTMs were processed further for RNA extraction. Viral RNA extraction from all collected samples was carried out using ***Aridia DNA/RNA complete extraction kit***, as per the protocols mentioned in manufacturer's kit literature, with a final elute volume of 40 µL. Once, RNA was extracted, it was transferred for analysis maintaining a temperature of -20°C. After RNA extraction real-time RT-PCR was carried out as per procedure mentioned below.

The real-time RT-PCR was done using ***TRUPCR®SARS-CoV-2 RT qPCR KIT (V-3.2).*** It uses the TaqMan fluorogenic probe-based chemistry that used the 5′ nuclease activity of Taq DNA polymerase and enables the detection of specific RT-PCR product as it is accumulated during the cycle in a real-time manner. Suspected conjunctival swab samples were tested simultaneously for presence of E gene, RNA-dependent RNA polymerase and N gene in a single assay. Positive control and negative control were also included in each test run.

Real-time thermocycler (***ALTA RT 96 real-time PCR by Athenese Dx. Pvt. Ltd.)*** calibrated for required dyes was prepared by setting up of program/temperature profile following settings as per kit literature provided PCR tubes containing PCR mix and template were loaded in a thermocycler for amplification and detection. Results were analyzed using a threshold cutoff value (Ct) of 35. Before proceeding for reporting of test results, QC (positive control reaction and negative control reaction) were checked for performance.

Statistical methods

Descriptive statistical methods such as mean were applied to variables, e.g. mean age, mean CT value with standard deviation in the study group. Fischer's exact test was applied as the test of significance to find out associations between conjunctival swab positivity and other factors such as comorbidity and severity. Cohen's kappa was used to determine the agreement between nasopharyngeal swab RT-PCR positivity and conjunctival swab RT-PCR positivity.

Results

We have enrolled 155 patients in our study, out of which 5 were excluded as samples of those were inadequate for analysis. Out of 150 patients, 86 were males and 64 were females. The mean age of patients was 51.68 (SD ± 14.43). Ninety-one patients (60.7%) had mild disease. In total, 39 patients (26%) had moderate disease and 20 patients (13.3%) were included in severe category. Out of 155 patients, percentage of patients with comorbidities was 51%. Number of patients with diabetes mellitus (DM) was 43. Patients with only hypertension (HTN) were 47 in number. Four patients had coronary heart disease and two patients had chronic kidney disease. Other comorbidities included hypothyroidism (8), rheumatoid arthritis (1), and asthma (2). Out of 150 patients, two patients were having conjunctival manifestations in the form of conjunctivitis. These two patients were negative for conjunctival swab RTPCR. Conjunctival swab RT-PCR was positive in five patients (3.33%), out of total 150 patients. Among % positive patients, two (40%) were from mild, one (20%) was from moderate, and two (40%) were from severe category. Two patients (40%) were having DM with HTN and rest three (60%) were having DM. All the positive patients were above 50 years of age, out of which four were 60 years and above. The mean Ct value of E gene in five positive patients was 30.4 (SD ± 4.82). The mean Ct value of RdRP gene was 29.8 (SD ± 6.22). The mean age of patients with positive conjunctival swab RT-PCR was 67.6 (range 58–85). Only one of the positive patient had a Ct value less than 25. Despite signifying high viral load, the patient had moderate disease on CT scan. No correlation could be established between the Ct value and the severity of disease.

Discussion

Arora *et al*.[11] have included 75 moderate to severe COVID-19 positive patients in their study. They collected tear samples through Schirmer tear test strip and taken conjunctival swab as well. Out of 75 patients, SARS-COV-2 was present in 18 (24%) of tear samples. In a study conducted by Zhang *et al*.[12] out of 72 COVID-19 patients, conjunctival swab RT PCR was positive in one tear sample. Similarly Fang *et al*. enrolled 32 COVID-19 patients. RT-PCR of Conjunctival swabs were positive in 5 samples (15.63%).[13]

Various reasons can be postulated for less percentage of conjunctival swab RT-PCR positivity in our study. This includes inclusion of mild and asymptomatic patients, single time sampling, and low sensitivity of RT PCR. A recent study on rhesus macaques stated that virus is present in ocular tissue for a short period of time as most of the fluid is either swallowed or drained in nasopharyngeal space.[14]

Statistical analysis using Cohen's kappa found a slight agreement between conjunctival swab RT-PCR and

nasopharyngeal swab RT-PCR [Table 1]. Hence, conjunctival swab RT-PCR alone cannot be relied upon for the diagnosis of SARS CoV-2 infection.

Conjunctival manifestations in the form of conjunctivitis were present in two patients (1.33%) in our study. Conjunctival swab RT-PCR was negative in these patients. Correlation could not be established between conjunctivitis and conjunctival swab RT-PCR positivity.

According to study conducted by Güemes-Villahoz N, *et al*.[15] SARS-CoV-2 RNA was present in two patients (5.55%) out of 36 COVID-19 patients. Conjunctivitis was present in 18 patients. Only one showed positive result among the 18 patients. Similarly in a study of 121 COVID-19 positive patients done by Zhou *et al*.[16] SARS-CoV-2 RNA were detected in conjunctival secretions of 3 (2.5%) patients. Conjunctivitis was present in eight patients out of which one was positive on conjunctival swab RT-PCR.

In our study, we performed sample collection within 48 h of admission to COVID-19 ward. This was the same as in the study by Arora *et al*.[11] Most other studies had sampling times at a mean of more than 3 days after admission.[12,16-18] It remains to be seen if time of sampling has an impact on the positivity rates due to variations in viral load over time.

Severity

Of the five patients with positive conjunctival swab, two (40%) had mild COVID-19 disease on the CT scan of chest, one (20%) had moderate disease, and two (40%) had severe disease. Wu *et al*.[7] reported 66% of conjunctival swab positive patients as severe/critical and 34% as moderate. Similar high rates of conjunctival positivity among severe cases were reported by Zhou *et al*.[19] and Karimi *et al*.[17] While in the study by Xia *et al*.[20] the only positive patient out of 30 was categorized as mild, Karimi *et al*.[17] concluded that severe patients had more likelihood of conjunctival positivity than nonsevere patients. This corresponds to our study where three out of five positive patients belonged to moderate to severe category. However, in our study, the association between severity and conjunctival swab positivity was found to be nonsignificant ($P = 0.18$) [Table 2].

Comorbidity

In our study, all patients who were positive for RT-PCR on conjunctival swab had comorbidities: All five had diabetes (100%); two patients (40%) had hypertension. No association was found between comorbidity and conjunctival swab positivity ($P = 0.06$). This is similar to the finding by Arora *et al*.[11] [Table 3].

Asymptomatic

In our study, five out of 150 (3.33%) patients were asymptomatic, but having changes suggestive of COVID-19 on CT scan of chest. None of these patients were positive on RT-PCR of conjunctival swab. Statistical analysis revealed no significant association between symptomatic/asymptomatic patients and conjunctival swab positivity ($P = 1.0$) [Table 4].

Our study is limited by relatively small sample size. The other limitation was single time sampling as repeated sampling may increase the chances of getting more positive conjunctival swab RT-PCR due to low sensitivity of RT-PCR.

Table 1: Conjunctival swab RTPCR

	Positive	Negative
Nasopharyngeal SWAB RTPCR		
Positive	5	145
Negative	0	0

Number of observed agreements: 5 (3.33% of the observations). Number of agreements expected by chance: 5.0 (3.33% of the observations), kappa=0.000, i.e., slight agreement

Table 2: Association between severity and RT-PCR findings

	RT-PCR Positive	RT-PCR Negative
Mild	2	89
Moderate	1	38.0
Severe	2	18.0
Total	5	145

From Fisher's exact test, P=0.18 (not significant), i.e., association between severity and RT-PCR findings is not significant

Table 3: Association between comorbidity and conjunctival swab RT-PCR positivity

	Conjunctival swab RT-PCR positive	conjunctival swab RT-PCR negative
Comorbidity present	5	72
Comorbidity absent	0	73

From Fisher's exact test, P=0.06 (Statistically not significant), i.e., association between comorbidity and conjunctival swab RT-PCR positivity is not significant

Table 4: Association between asymptomatic/symptomatic patients and conjunctival swab RT-PCR positivity

	Conjunctival swab RT-PCR positive	conjunctival swab RT-PCR negative
Asymptomatic	0	5
Symptomatic	5	140

From Fisher's exact test, P=1.00 (statistically not significant), i.e., association between asymptomatic/symptomatic patients and conjunctival swab RT-PCR positivity is not significant

Conclusion

In conclusion, our study supports and provides evidence that though incidence of SARS-CoV- 2 virus in conjunctival secretions is very low, eye may act as source of transmission. In addition, SARS-CoV-2 virus can inoculate through tears and conjunctival secretions and further travel to nasopharynx through a nasolacrimal drainage system. Hence, extra caution should be taken by healthcare workers specifically by ophthalmologists while examining patients in outpatient departments and use of proper precautions like face shields and goggles should be enforced. Further studies may be needed with a large sample size and multiple samplings to throw light and give better understanding of ocular route of transmission of SARS-CoV-2 virus.

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Conflicts of interest

There are no conflicts of interest.

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