Conjunctival Swab Positivity and Conjunctivitis in SARS-COV-2 in a Tertiary Care Center of Western Odisha

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ABSTRACT

Introduction: The principal route of transmission of severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) is respiratory droplets. Reverse transcription-polymerase chain reaction (RT-PCR) test of throat swabs, nasopharyngeal swabs, sputum, endotracheal aspirates and bronchoalveolar lavage is the diagnostic test of severe acute respiratory syndrome coronavirus-2. Since the epithelium of the conjunctiva contains angiotensin-converting enzyme-2 receptors, the presence of the severe acute respiratory syndrome coronavirus-2 in conjunctival secretion can be expected. The present study is designed to detect severe acute respiratory syndrome coronavirus-2 from conjunctival secretions and the prevalence of conjunctivitis in laboratory-confirmed CoronaVirus disease-19 (COVID-19) patients.

Materials and methods: This is a prospective observational study carried out in a tertiary care hospital in western Odisha, India from September 2020 to November 2020 during the first wave of CoronaVirus disease-19. One hundred and thirteen laboratory-confirmed CoronaVirus disease-19 positive patients either by reverse transcription-polymerase chain reaction or Rapid antigen test (RAT) from nasopharyngeal swabs were included. Conjunctival swabs were collected from all these patients with proper precautionary measures and sent for reverse transcription-polymerase chain reaction test. Any signs of conjunctivitis at any stage of the illness were observed in all the patients.

Results: Out of 113 samples, reverse transcription-polymerase chain reaction test of the conjunctival swab was found to be positive in three patients (2.65%). The mean cycle threshold (CT) value of these three swabs was 27.16. No signs of conjunctivitis were found in any of these patients. Diabetes and hypertension were associated comorbidities in one patient.

Conclusion: The absence of conjunctivitis despite the presence of virus in the conjunctival swab gives a message to the ophthalmologists to take precautionary measures during a routine eye examination.

Key words: Conjunctival swab, Conjunctivitis, COVID-19, RT-PCR test, SARS CoV-2.

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INTRODUCTION

Coronaviridae is a well-recognized and notorious family of viruses that cause many diseases ranging from the common cold with mild symptoms to potentially life-threatening human respiratory infections such as the Middle East respiratory syndrome and severe acute respiratory coronavirus syndrome (SARS-COV) (Weiss and Navas-Martin, 2005). The SARS-CoV-2 virus is a novel coronavirus and it emerged in the Hubei province of China in December 2019. On 11th March 2020, the World Health Organization(WHO) proclaimed the novel coronavirus epidemic a pandemic, thereby raising the likelihood that the virus will spread to all countries around the world(Liu et al., 2020). As per the data by WHO, there are 35.6 crores of COVID-19 worldwide with 56.1 lakhs deaths by January 2022 (World Health Organization. WHO coronavirus (COVID-19) dashboard. World Health Organization. Retrieved January 27, 2022, from https://covid19.who.int/). India, the 2nd largest populated country, contributes to about 4 crores confirmed cases with 4.91 lakhs deaths (World Health Organization. (n.d.). India: WHO Coronavirus disease (COVID-19) dashboard with vaccination data. World Health Organization. Retrieved January 27, 2022, from https://covid19.who.int/region/searo/country/in).

The principal route of transmission of SARS-COV-2 is direct contact with respiratory droplets of infected people. Additionally, the transmission can also occur when a person touches a virus-contaminated surface or object and then touches his or her mouth, nose, or eyes(Huang C et al., 2020). The common signs and symptoms of COVID-19 include fever, cough, shortness of breath, myalgia, weakness, sputum production, headache, hemoptysis, diarrhoea and conjunctivitis (Wu et al., 2020). The most common ocular manifestation documented till now is acute conjunctivitis (P. Zhou et al., 2020).

The coronavirus is the largest RNA virus in base length and is divided into 4 groups: α-,β-, γ-, and δ-coronavirus (Oade et al., 2019). The SARS CoV-2, which belongs to the beta coronavirus have almost 90% of sequence identity in their spike protein S2 subunits which help in the membrane fusion process and the S1 subunits utilize the human angiotensin-converting enzyme 2 (hACE2) as the receptor to infect human cells(Bosch et al., 2004). The S-Protein of the virus gets attached to the ACE-2 receptor in the target cell following which heptad repeat 1(HR1) and the heptad repeat 2(HR2) in the S2 subunit interact with each other and form a six-helix bundle (6HB) fusion core bringing the viral and cellular membrane close aiding fusion and infection. The virus enters the host cell cytosol by the action of cathepsin protease by proteolytic cleavage of the S protein. The fusion occurs in the endosomes forming the bundles and the viral genome is released in the host cell(Zhang et al., 2020).

With this background knowledge of SAR-COV-2, the objective of the present study is to estimate the percentage of SARS-CoV-2 detected from the conjunctival swab and the prevalence of conjunctivitis among laboratory-confirmed COVID-19 patients

MATERIALS AND METHODS

This was a prospective observational study carried out from September 2020 to November

2020 in a tertiary care hospital in western Odisha, India during the first wave of COVID-19. It included 113 laboratory-confirmed COVID-19 patients who were either RT-PCR or Rapid antigen test (RAT) positive for SARS CoV-2 in the nasopharyngeal swab. Conjunctival swabs were collected from all these patients with proper precautionary measures and sent for RT-PCR test All of them were admitted either to the isolation ward or COVID hospital. Critically ill patients could not be included in the study because the required consent could not be obtained as the patients were not able to give consent and no family members were allowed to accompany the patients to give consent on behalf of the patient during that period as per prevailing guidelines. Simple random sampling was the method for the selection of the patients.

The study was started after approval from the Research Ethics Committee of the institute. Confidentiality and anonymity were maintained throughout the study by coding data and were kept in security. There were no risks or adverse effects to the study population. No compensation (monetary or non-monetary) was given to participants.

A proper history including the age, sex, symptoms, duration of illness, and any pre-existing/underlying comorbidities like diabetes mellitus, hypertension, coronary artery disease, and acute/chronic kidney disease was noted. After wearing the personal protective equipment, N95 mask, gloves and face shield, an ophthalmologist posted at COVID hospital collected the conjunctival swab. Under proper aseptic measures, the lower lid was gently pulled down and the conjunctiva was exposed. The swab was gently swiped along the lower fornix from inner to outer canthus and the same procedure was repeated in the other eye. After collection, the swab was dipped into 2-3 ml of the viral transport medium (VTM) and was properly sealed and labeled. It was transported to the Viral Research Diagnostic Laboratory (VRDL) of Regional Medical Research Center-Indian Council of Medical Research (RMRC-ICMR), Bhubaneswar under refrigerated conditions after triple-layer packaging. If a delay in testing or shipping is expected, specimens were stored at -70°C or below in the COVID testing lab of the microbiology department of the hospital.

Sample processing

Once the samples reach the VRDL of RMRC-ICMR, Bhubaneswar, with all standard safety protocols of WHO, the sample processing was done in a class-II biological safety cabinet. For nucleic acid extraction before RT PCR, the specimen was transferred to a lysis buffer under the Biosafety Level 2 (BSL-2) cabinet. The lysis buffer contains a guanidinium-based inactivating agent as well as a non -denaturing detergent. RNA was extracted using the QIAamp virus RNA mini kit (Qiagen) as instructed by the manufacturer and cDNA was produced according to the standard protocol as described by the manufacturer.

Real-time qualitative PCR assay for SARS-CoV-2

The TaqPath COVID-19 Combo Kit was used with the Applied Biosystems 7500 Fast Dx Real-Time PCR System and Applied Biosystem COVID-19 Interpretive Software The kit contains TaqPath™ 1-Step Multiplex Master Mix, Real-Time PCR Assay Multiplex, and

Nuclease-free Water which was ready to use for RT-PCR. First, the reactions were incubated at 50 ^{0}C for 2 minutes, followed by appropriate thermal cycling conditions (45 cycles) according to the kit insert. Then the result was analyzed after 1.30 hour of cycling conditions in the ABI biosystem.

The positive results were analyzed and a proper record was maintained. The quantitative values of data analysis were expressed as mean, frequency, and percentage. Data entry was done by statistical software: SPSS version 23/ MS EXCEL. The percentage of positivity for SARS CoV2 in conjunctiva was calculated.

RESULTS

Out of 113 COVID-positive patients from whom conjunctival swab was collected, 84(74.3%) were males and 29(25.6%) were females with a male to female ratio of 2.8:1. The mean age of the patients was found to be 49 years.30.9 % of patients were in the age group of 51 to 60 years.

The nasopharyngeal swab of the patients was Rapid Antigen Test (RAT) positive in 72 patients (63.8%) and RT-PCR test positive in 41(36.2%). Various comorbidities associated with the patients are described in Table 1. Diabetes mellitus (22.1%) and hypertension (20.3%) were found to be the two most common associations.

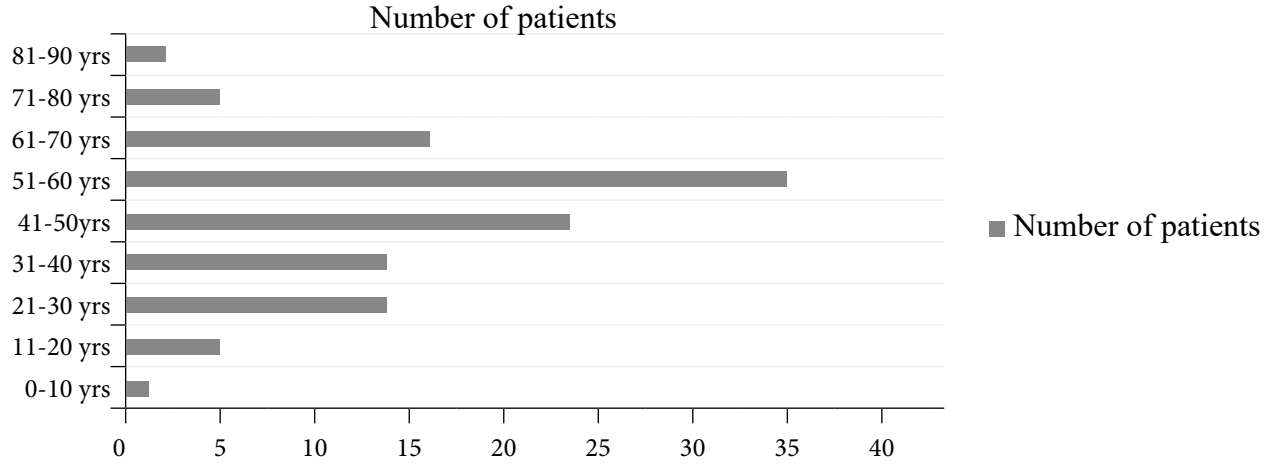

Figure 1: Age groups of all the patients included in the study.

Table 1: Various associated comorbidity.

Comorbidities	Total number of patients(n=113)	Percentage
Diabetes mellitus	25	22.1%
Hypertension	23	20.3%
Chronic kidney disease	6	5.3%
Coronary artery disease	3	2.6%
Bronchial asthma	2	1.7%
Cerebral Vascular Accident	1	0.8%
Chronic obstructive pulmonary disease	1	0.8%
Sickle cell disease	1	0.8%

Table 2: Various manifestations in COVID patients.

Symptoms	Total number of patients (n=113)	Percentage
Fever	43	38.0%
Shortness of breath/Dyspnoea	32	28.3%
Cough	21	18.5%
Coryza	14	12.3%
Asymptomatic	13	11.5%
Severe Acute Respiratory Infection	13	11.5%
Pain abdomen	6	6.1%
Admitted for other reason	10	8.8%

Table 3: Details of patients who were RT-PCR positive for conjunctival swab.

Patient	Age	Sex	RT-PCR / RAT positive for Nasopharyngeal swab	Symptoms	Comorbidities	Treatment given
Patient A	40	Male	Rapid antigen test positive	Fever Seizure	None	Inj Piperacillin+tazobactam, Inj paracetamol Inj methylprednisolone Inj Pantoprazole Inj Enoxaparin Inj Remdesivir Intubation
Patient B	50	Male	Rapid antigen test positive	Cough	Diabetes mellitus Hypertension	Inj piperacillin+tazobactam Inj paracetamol Inj methylprednisolone Inj Pantoprazole Inj Enoxaparin Inj Remdesivir Inj Human Insulin
Patient C	45	Male	Rapid antigen test positive	Diarrhoea	None	Tab Ofloxacin Tab metronidazole Tab Hydroxychloroquine Tab Zinc Tab Vitamin C Tab Ivermectin Tab Pantoprazole Oral rehydration Salt

The different manifestations found in COVID patients are listed in Table 2. Fever (37.1%) was the most common symptom followed by shortness of breath (28.3%).

RT-PCR test of the conjunctival swab was found to be positive in three patients (2.65%). The mean cycle threshold (CT) value of these three swabs was 27.16. The details of these three patients are given in Table 3. The nasopharyngeal swab of all of these 3 patients was RAT (Rapid Antigen Test) positive. No signs of conjunctivitis were found in any of these three patients. All three patients were males and above the age of 40 years. One of these patients had an association with Diabetes and hypertension and the other two did not have any comorbidity. Out of 3 patients, two patients had received injectable antiviral Remdesivir along with other drugs when required as per the prevailing guideline during that time.

DISCUSSION

SARS-CoV-2 is a highly transmissible virus and the modes of its transmission are respiratory droplets and contact with infected persons or objects. The shedding of the virus through alternative routes like ocular secretion or tears can be possible as the conjunctiva contains the ACE receptor in its epithelium. The studies about the presence of the SARS-COV-2 virus in conjunctival secretion are very few to date. The comparison of conjunctival swab positivity and prevalence of conjunctivitis in SARS-COV-2 in different studies is given in Table number-4. In the present study, the presence of viral RNA in conjunctival secretion was found to be 2.65% which is consistent with different studies conducted in India and China(Kumar et al., 2020; Y. Zhou, Duan, et al., 2020). However, one study by Arora et al from India found that the RT-PCR positivity of conjunctival swabs is as high as 24%(Arora et al., 2021). Arora et al concluded that the high detection rate of SAR-COV-2 in their study may be due to the inclusion of moderate and severe disease, tear sample collection early in the course of the disease and inoculation of samples from both eyes.

The viral load of COVID-19 patients is determined by the cycle threshold value (CT value) of the RT-PCR test. CT value is the number of cycles at which fluorescence of the Polymerase chain reaction (PCR) product is detectable over and above the background signal. CT value is inversely related to the amount of SAR-COV-2 RNA in the starting sample and the lower the CT value higher the viral load(Corman et al., 2020). It has been observed that there is a 10-fold reduction of viral load for a 3.3 increase in CT value The mean CT value of the RT-PCR test of the conjunctival swab in the present study was found to be 27.1 which is quite low as compared to Chowhan et al who found it to be 30.4 in their study(Chawhan et al., 2021). This indicates that the viral load in the three positive samples in our study was quite high. The relationship between disease severity and viral load is differently reported by different authors. Some researchers reported that the more the viral load, the more is the disease severity (Fajnzylber et al., 2020; Liu et al., 2020; Magleby et al., 2021). While Lee et al showed that there was no significant difference between viral load and clinical course (Lee et al., 2020), Argyropoulos et al. demonstrated viral load of indoor patients with the disease was lower than in outdoor patients(Argyropoulos

et al., 2020). Furthermore, Zou et al. showed that the viral load between symptomatic and asymptomatic cases was similar (Lirong Zou, M.Sc. Guangdong Provincial Center for Disease Control and Prevention Guangzhou et al., 2020).

Ziad and colleagues found that tracheal aspirates have significantly higher SARSCoV loads compared to the nasopharyngeal swab and sputum specimens(Memish et al., 2014). This suggests that the viral concentration may be different at the various sites of the human body. In consideration of the fact that the ocular surface is an open microenvironment, and that the virus may transport to the inferior meatus of the nose rapidly, the SARS-CoV2 concentration on the ocular surface is likely to be very low irrespective of the disease severity(Paulsen, 2007). This may be one of the reasons for less positivity in the conjunctival swab sample.

Though SAR-COV-2 was isolated in 3 conjunctival samples, we did not find any signs of conjunctivitis in them which is consistent with many studies(Kumar et al., 2020; Arora et al., 2021; Li et al., 2021). The ocular manifestations in the SAR-COV-2 virus were found in 31.6% and 25 % by Wu P et al and Atum et al (Atum et al., 2020; Wu et al., 2020) respectively although they have found less number of samples of conjunctiva being positive with RT-PCR. This suggests that the mere presence of the virus in conjunctival secretions does not always imply a cause of conjunctivitis in COVID patients.

It has been postulated that after direct inoculation of infectious droplets, the conjunctiva may serve as a route for viral entry through the nasolacrimal duct to the nasopharynx, but is protected from direct viral invasion by the constant flushing of the tear film and due to the action of antimicrobial peptides and immunoglobulins in human tears (Hong et al., 2020).

Savastano et al concluded that an almost complete absence of conjunctival inflammatory reaction in conjunctival swab-positive patients implies good conjunctival tolerability in the presence of the virus, and is probably linked to a low local antigenic reactivity (Savastano et al., 2021). This might be the probable reason for the absence of conjunctivitis despite swab positivity in the present study

Further, it has been reported that conjunctivitis has been associated with a more severe form of COVID-19 disease (Loffredo et al., 2020). Hyperinflammatory response induced by SARS-CoV-2 is a major cause of the disease severity (Merad and Martin, 2020). Therefore, conjunctivitis in patients with COVID-19 could represent an inflammatory response to the disease, manifested by inflammation of the conjunctiva. The exclusion of critically ill patients where the probability of inflammatory response is high explains the absence of conjunctivitis in the present study.

Xia et al in their study isolated the SARCOV-2 virus in a conjunctival swab of only one COVID-19 patient with conjunctivitis out of 30 patients (Xia et al., 2020). They have reported that the use of antiviral treatment by the patient may be one of the important factors for their result. In the present study, injectable remdesivir was administered to two out of three patients where the conjunctival sample was positive. So though the effect of antiviral treatment on conjunctivitis could not be excluded in the

Table 4: RT-PCR test of conjunctival swab and Incidence of conjunctivitis in different studies.

Author	Year	Place	Number of Laboratory confirmed COVID patients	RT-PCR positive in conjunctival swab	% of positivity	Incidence of conjunctivitis	%
(Kumar et al., 2020)	2020	India	45	1	2.22	0	0
Xia et al	2020	China	30	1	3.33	1	3.33
Atum et al.	2020	Turkey	40	3	7.50	10	25.0
Y.Zhau et al.	2020	China	121	3	2.47	8	6.61
Wu et al	2020	China	38	2	5.26	12	31.5
Zhang et al	2020	China	72	1	1.38	2	2.77
Li et al	2021	Hongkong	49	4	8.16	0	0
Arora et al	2021	India	75	18	24.0	0	0
Present study	2020	India	113	3	2.65	0	0

present study, a more comprehensive study with larger sample size is needed to prove the fact.

Fever, respiratory symptoms, seizures and diarrhoea were the systemic symptoms associated with our conjunctival swab RT-PCR positive patients. But Li X et al reported that 50% of their patients suffered from fever and 50% suffered from respiratory systems (Li et al., 2021). Diabetes mellitus and hypertension were associated in 1 out of 3 patients with RT-PCR positive for conjunctival samples (33.3%) in the present study which is consistent with Arora et al who have documented more than one associated comorbidity in 6 out of 18(33.3%) patients (Arora et al., 2021). The mean age of patients who are positive for RT-PCR in conjunctival secretion was found to be 45 years in this study. But Arora et al have found it to be 56.78±16.79 years. All three patients were male in our study in contrast to other studies where both males and females were found (Arora et al., 2021; Li et al., 2021). The comparison of the present study to other similar studies is described in Table 4.

CONCLUSION

The present study shows that the SAR COV-2 virus can be isolated from conjunctival samples of laboratory-confirmed COVID-positive patients. Though the positivity rate is very less as compared to other body secretions, the transmission of the virus through tears cannot be denied. Further, the absence of conjunctivitis even in presence of the virus in conjunctival swabs may be due to host immune factors like constant flushing of the tear film, antimicrobial peptides and immunoglobulins in human tears.

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