ORIGINAL ARTICLE

SARS-COV2 Virus in Tears and Conjunctival Secretions of COVID-19 Patients

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ABSTRACT

Purpose: To determine the presence of SARS-COV 2 viruses in tears and conjunctival secretions of patients with diagnosed COVID-19.

Study Design: Prospective interventional case series.

Place and Duration of Study: Rawalpindi Institute of Urology, Department of infectious diseases, Holy Family Hospital, Rawalpindi Medical University, from September 2020 to October 2020.

Methods: Samples of conjunctival and tear secretions were collected from 60 hospitalized patients of COVID-19 who were confirmed with nasopharyngeal swabs test. Disposable conjunctival swab sticks were used for sampling. Samples were taken within 3 days of admission. SARS-COV 2 virus evaluation in tears and conjunctival secretions was done by Qualitative RT-PCR (Reverse transcriptase polymerase chain reaction) analysis. Ocular features were documented. Regarding systemic course of illness, details were noted from their hospital records.

Results: Mean age of the patients was 56.63 ± 16.373 years. Out of 60 patients, 42 were males and 18 were females. Twenty two (36.6%) patients had mild disease and moderate and severe disease was present in 19 patients (31.6%) each. Six (10%) patients had positive conjunctival and tear secretions for SARS-COV 2 viral RNA. All patients with positive ocular secretions for viral RNA were in first week of course of disease and 3 patients had severe COVID 19 disease signifying high viral load. Only one patient had conjunctivitis and ocular symptoms of redness and foreign body sensation.

Conclusion: There is likelihood of SARS-COV 2 virus transmission via ocular secretions as frequency of patients with SARS-COV 2 viral RNA detected in tears was 10% in current study therapeutic services.

Key Words: SARS-COV 2 Virus, COVID 19, Conjunctivitis, Qualitative RT-PCR, Ocular Manifestations.

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INTRODUCTION

SARS-COV-2 virus causing corona virus disease had spread worldwide posing a massive threat to health, while viral transmission routes are still debatable. Current pandemic of COVID-19 caused by SARS-COV 2 virus has affected 223 countries and territories with total of 91 Million cases and a mortality of 1.97 Million, posing a massive threat to health globally. Corona viruses are RNA positive, non-segmented enveloped viruses having crown like spikes.[1] They belong to large family of Coronaviridae with genera of alpha, beta, gamma and delta. Among Beta corona viruses, SARS-COV caused SARS (Severe Acute Respiratory Syndrome) pandemic in 2002-4 and MERS-COV caused pneumonia pandemic of MERS (Middle East Respiratory Syndrome) in 2012 causing a

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mortality of 10% and 37% respectively.[2] COVID-19 initiated as cluster of pneumonia cases outbreak in Wuhan, China December, 2019 and this novel pathogen was detected as type of corona virus.[3] In February 2020, this virus was officially named as SARS-COV2 (2019-Ncov) by World Health Organization.[4] Novel corona virus disease primarily has flu like symptoms of fever, cough along with shortness of breath in severe cases and an incubation period of 2 – 14 days. The illness severity ranges from asymptomatic in some individuals while others develop mild to severe disease and even death.[5] COVID-19 is extremely communicable disease and it is spread by either direct or indirect contact with respiratory droplets, secretions and fomites of infected people.[6]

Transmission of COVID 19 via ocular secretions is still controversial. SARS-COV 2 uses the same receptor as SARS-COV, the Angiotensin Converting Enzyme 2(ACE 2) receptor to hook into epithelial cells with cilia and pneumocytes.[7] SARS COV was reported in tears of patients in 2004 pandemic. Conjunctival and corneal epithelial cells express ACE 2 receptors and TMPRSS2 protease genes (type II Trans Membrane Serine Protease).[8] Both are necessary for viral entry into cells and portal for transmission from person to person. Hence, route could be infection of lacrimal gland via hematogenous spread resulting in shedding of virus in tears or from respiratory tract to lacrimal drainage system via nasolacrimal duct.[9,10] Ocular infection could also be due to direct inoculation of cornea and conjunctival epithelial cells by virus. Ophthalmologists are at greater risk due to close proximity to patients while examining and performing diagnostic and therapeutic procedures. Multiple case reports depicted that ophthalmologists acquiredCOVID-19 during diagnostic and therapeutic services.[11] It is speculated that exposure to respiratory secretions is not the only way of viral spread. This fact explicates that unprotected eyes leading to exposure of conjunctiva and cornea to aerosols can be a source of viral transmission.[12] Consequently, probability of ocular secretions being a gateway for infection spread is still questionable. There has been an increasing receptiveness and curiosity in the means of viral spread equally for medical concerns and public health awareness allegations.

The current study was conducted to determine the frequency of SARS-COV 2 viruses in ocular secretions of patients with confirmed COVID-19 and its effectiveness as diagnostic tool for COVID-19 infection. Correspondingly, to determine whether all or only conjunctivitis patients with confirm COVID-19 have positive virus in their ocular secretions.

METHODS

It was a Prospective interventional case series of 3 months duration conducted from September 2020 to October 2020. Sample size for the study was 60, by WHO sample size calculator, keeping Level of confidence as 95% with Margin of error as 6.5%. Sampling was done by Non-probability consecutive sampling technique. 60 hospitalized COVID-19 patients at Rawalpindi Institute of Urology and Department of infectious diseases DID, Holy Family Hospital, Rawalpindi Medical University (Multicenter trial) meeting the bench mark were enrolled. Patients ranging from mild to severe disease were included. Mild disease was defined as patients maintaining oxygen saturation at room temperature of 95%. Moderate disease was defined as oxygen saturation < 95% at room temperature, respiratory rate > 26cycles /min and heart rate > 126/min. Severe disease included patients in Intensive Care Unit, requiring assisted ventilation, in shock or multiple organ failure. Inclusion criteria was patients with positive RT-PCR analysis for corona virus nucleic acid in nasopharyngeal samples or positive CT chest findings of unilateral or bilateral patchy multi lobar lungs infiltration (typical ground glass appearance) detected by radiologist as viral pneumonia. Patients less than 18 years of age or not willing for consent were excluded from the study. Patients with suspected or symptomatic disease but negative RT-PCR or CT chest findings were also excluded.

After approval by ethical review board (ERB) and institutional research forum (IRF) the study followed the tenants of Helsinki declaration. Detailed informed consent was taken from all patients. Ocular secretion samples were collected within first three days of admission of confirmed COVID-19 cases. Sampling was done by resident ophthalmologist with proper Personal Protective Equipment donning via conjunctival swab technique. Conjunctival swab stick was swiped into lower conjunctival fornix of either eye for 15 secs by asking patient to look in the upward direction and pulling the lower lid down without any local anesthetic. Swab stick was placed into VTM tube containing the viral transport media and other half of

stick was broken to cap the tube. The samples were stored in ice packs and then sent for analysis in laboratory. Gloves were changed after every sampling to avoid cross infection and contamination of samples. Samples analyses for viral RNA were done by qualitative Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) in pathology department of holy family hospital.

At time of collection of samples, demographic details including age, gender, symptoms of COVID 19 like fever, dry cough, and shortness of breath were documented. Time duration of systemic and ocular symptoms and disease severity was also entered in proforma. Ocular symptoms include watering, discharge, redness, blurring of vision and signs of conjunctivitis was noted. Positive test for viral RNA isolation was based on gene detection by qualitative RT-PCR analysis.

Data analysis was done by SPSS version 21. For quantitative variables like age, duration of systemic symptoms mean with standard deviation was calculated. For qualitative variables like gender, COVID 19 symptoms, ocular symptoms, disease severity, qualitative RT-PCR of ocular secretions, frequency (percentage) was calculated. Pearson's chi-square test was used to test the statistically significant association between categorical variables. p-value < 0.05 was considered statistically significant.

RESULTS

Mean age of patients was 56.63 years SD ± 16.373 with a range of 18 – 89 years. There were 42 males (70%) and 18 females (30%). Thirty five ocular secretion samples (58.3%) were collected from admitted patients of Department of Infectious Diseases, Holy Family Hospital and 25 samples (41.7%) from Rawalpindi Institute of Urology. Right eye was tested in 32 (53.3%) cases and Left eye in 28 (46.7%) cases.

Among COVID 19 symptoms, fever was present in 59 cases (98.3%), cough in 57 cases (95%) and dyspnea in 30 (50%) patients. Out of 60 patients, 21 (35%) had mild disease, 19 (31.7%) had moderate disease, 20 (33.3%) had severe disease. Among patients with severe disease, noninvasive ventilation was required in 5 (8.33%) patients and invasive Ventilation via endotracheal tube in 6 (10%) patients. CT scan findings showed patchy lobar infiltration in 18 (30%) patients, ground glass appearance in 23 (38.3%) patients and both in 19 (31.7%) cases. Mean duration of systemic symptoms was 9.00 days ± 2.762 SD with a range of 2 – 14 days.

Only one patient had signs of conjunctivitis. No ocular manifestations were seen in 59 (98.3%) cases. Qualitative RT-PCR of 6 (10%) patients tested positive for viral RNA isolation. Out of these 6 patients, 3 cases had severe disease, 1 case had mild disease and 2 cases had moderate disease.

All 6 patients who tested positive for RT-PCR had first week of course of disease (sampling done within 7 days of onset of symptoms) which was statistically significant p value < 0.05. Only 13 (21.6%) samples were taken during first week of course of disease as shown in figure 1.

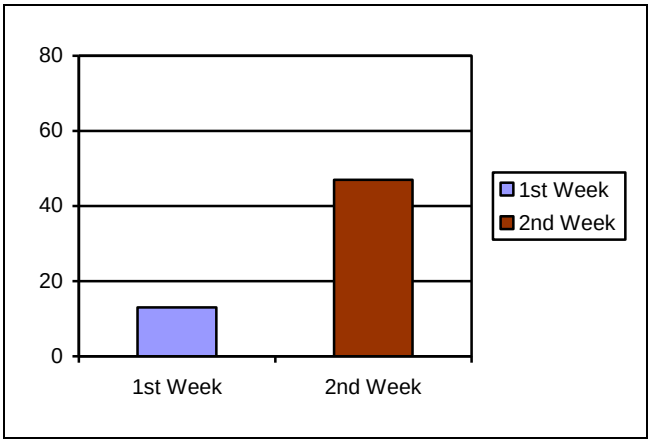

Figure 1: Samples Collected In Different Course of Disease.

No statistically significant relationship was seen between age, gender, disease severity and positivity of ocular secretions for RT-PCR (p value > 0.05). Conjunctivitis patient's ocular secretions were negative for viral RNA. All 6 samples tested positive for viral RNA had no ocular manifestations. All positive RT-PCR ocular secretion samples were also positive for nasopharyngeal RT-PCR.

DISCUSSION

Owing to few reports, there is uncertainty in viral detection and shedding only in ocular secretions of conjunctivitis patients or not. Though ocular transmission and detection of SARS-COV2 virus in tears and conjunctival secretions has not been confirmed and is still debatable, current study detected SARS-COV 2 viral RNA by qualitative RT-PCR in

tear samples of 6 patients with confirmed COVID-19.SARS-COV 2 enters the pneumocytes via Angiotensin converting enzyme 2 receptors (ACE 2). Shengjie Li et al reported that diseased conjunctiva expresses increased ACE 2 protein receptors as compared to healthy conjunctiva. Therefore, either direct inoculation, spread via hematogenous route or nasolacrimal duct can be source.[9,12]

Colavita et al reported detection of viral RNA in conjunctival secretions of first confirmed case in Italy in January, 2019. Patient had conjunctivitis and real time RT-PCR of conjunctival swab tested positive. The results stayed positive till day 21 and on day 29, viral RNA load was higher than nasal swabs.[13] Scalinci SZ et al reported five patients of non-remitting conjunctivitis who were confirmed as COVID-19 positive but conjunctival secretions were not evaluated for corona virus.[14] Xia J et al did study on 30 patients; they collected samples from either eye of COVID-19 patients. Out of these, 21 patients had mild and 9 patients had severe disease. Two tear samples had positive viral RNA from common type disease patient and that patient also had conjunctivitis. Rest of 58 samples were negative.[15] Mean age of patients was 54.50 years which were closer to mean age group of our sample. Males to females ratio was higher as compared to present study. It was reported by Xia J et al that patients without ocular manifestations of conjunctivitis were not a source for infection. Khavandi S et al reported positive ocular secretion for RT-PCR analysis in COVID-19 patient with conjunctivitis.[16] All of these studies demonstrated that conjunctival secretions of COVID-19 patients with conjunctivitis are more likely to have SARS-COV2 viral RNA. However, current study detected viral RNA in ocular secretions of patients without any ocular manifestations.

Zhou Y et al tested 67 patients' tears samples. One patient tested positive and 2 patients were probably positive. None had ocular symptoms. Only 1 patient had ocular findings but her tear samples tested negative.[17] They concluded low incidence of ocular transmission but nosocomial infection in health care workers can occur. Limitations were of sampling time lag. Zhou et al in another study tested conjunctival swabs of 121 covid-19 patients. Out of these, 113 had no ocular manifestations and 2 patients among these had positive viral RNA in their ocular secretions. Eight patients had conjunctivitis and among them, only one patient with severe disease was positive for viral RNA. Wu P et al conducted a study on 38 COVID 19 patients, 12 out of them had ocular findings of conjunctivitis, more frequent in severe patients with low prevalence. Only 2 patients had positive SARS-COV2 in tears.[18] Karimi S et al reported 7% positive tear samples (3 out of 43) and only one patient had conjunctivitis. Patients with conjunctivitis had positive tear samples when tested for viral RNA by RT-PCR.[19]

Most common reported symptom followed by shortness of breath and cough.

Zhang X et al reported only one patient with positive SARS-COV 2 by RT-PCR in ocular secretions, out of 72 COVID-19 tested patients. Two patients had conjunctivitis but none of them tested positive.[20] In another study, two covid-19 patients with conjunctivitis were reported by Zhang et al, one of whose swabs tested positive for viral RNA. Xie HT et al reported testing of tear secretions of 33 patients, out of them 2 showed positive results for SARS-COV2 viral RNA, both patients were without conjunctivitis.[21] Huang et al tested conjunctival secretions of 37 patients for SARS-COV-2 viral RNA by RT-PCR. Of these, 12 patients had severe symptoms and rests were mild. One patient with severe disease was tested positive for SARS-COV-2 viral RNA in conjunctival secretions by real-time RT-PCR but had no conjunctivitis.[22]

This gives us a clue that tear positivity for viral RNA is not due to conjunctivitis but it is also related to viremia thus patients with severe disease and high viral load are more likely to shed virus in their tears. All 6 patients who tested positive for RT-PCR in the current study had first week of course of disease, signifying high viral load within 7 days of onset of symptoms. Current study also demonstrated that SARS-COV 2 virus could be detected in tears and conjunctival secretions of patients without conjunctivitis.

There have been negative tests reports for ocular secretions when Schirmer test strips were used for viral isolation by qualitative RT-PCR testing.[23] Saeh IYJ conducted a study on 17 patients. One patient had ocular manifestations and all samples were negative for RT-PCR by Schirmer strips.[23] Hence, transmission via ocular route was not much favored by this data. They used Schirmer strips for sampling. Schirmer strips collect a small number of cells and secretions from conjunctiva, which could be the reason for false negative results. Hence, negative tests of tears and

secretions do not propose little risk for COVID-19 infection transmission. In Wuhan, China detection of SARS-COV 2 virus in 114ocular samples was done in an observational study by Deng Chao et al.[24] All samples were negative and none of the patients had conjunctivitis.

Conversely, compared to previous studies, current study reported high positivity rate of SARS-COV 2 viral RNA in ocular secretions (10%). Reasons for high positivity in this tested population could be due to sampling done from two different setups (multicenter trial) and only from admitted patients. Samples were collected within three days of admission and for a maximum of 2 weeks post COVID-19 symptoms to cover maximum viral load. Sampling was done by swiping the swab stick into lower conjunctival fornix for longer duration 15 sec without use of any topical anesthetic as topical anesthetic has dilution effect and can decrease viability of virus. There could be racial along with virus subtype differences.

Viral shedding in tears and secretions is for limited duration as tears are infected only in early course of disease. There could also be inadequate tears sample collection and few cells tested via conjunctival swab technique too. Calcium alginate swab sticks can cause virus deactivation. Negative results of the study can be partly attributed to viral load in nasopharyngeal secretions being higher than in ocular secretions. Low positivity rate in current study could also be due to this sampling being done in different courses of disease. As only 21.6% samples were taken from COVID-19 patients who had first week of course of disease. 78.4% samples were taken fromCOVID-19 patients who had more than 1 week of onset of symptoms. Qualitative RT-PCR kits have low sensitivity rate of less than 100% which was also a diagnostic dilemma in SARS virus evaluation.

Limitations of our study were small sample size. Furthermore, multiple samples from same patient in different course of disease need to be taken. Subsequently large sample size along with multiple samples from single patients should be experimented to confirm ocular transmission.

We speculate that SARS COV 2 viral RNA in ocular secretions of confirmed COVID 19 patients could be spotted whether patients are with or without conjunctivitis. We contemplate that eyes are not chief organs to disseminate the virus other than to ophthalmologists. Moreover, eyes themselves cannot produce infectious aerosol. However, eyes can be an imperative portal of entry of virus so they should be given enough consideration in PPE by providing water tight goggles. Ophthalmologists need to sanitize their hands and avoid hand-eye contact during routine outdoor procedures. Aerosol generating procedures like non-contact tonometry and contact equipment's like Goldman Applanation Tonometer, Argon laser photocoagulation, Confocal microscopy and Ultrasound Bio microscopy, there are high chances of cross infection hence they need frequent disinfection with antiseptics. Therefore, ocular transmission of SARS-COV2 viral RNA should be considered.

CONCLUSION

Likelihood of SARS-COV 2 virus transmission via ocular secretions is present as frequency of SARS-COV 2 virus in conjunctival secretions and tears was 10 % in current study. Severe/critically ill patients with high viral load and patients in first week of course of disease are more likely to shed viruses in their ocular secretions. Detection of SARS-COV 2 viral RNA in tears and conjunctival secretions could be seen whether patients are having conjunctivitis or not. Eyes can be an imperative portal of entry of virus so they should be given enough consideration in PPE by providing water tight goggles. Ocular protection can be an important precaution particularly for medical personals.

Ethical Approval

The study was approved by the Institutional review board/Ethical review board.
(Ref No. 131/IREF/RMU/2020)

Conflict of Interest

Authors declared no conflict of interest.

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Authors' Designation and Contribution

Ambreen Gul; Assistant Professor: *Concepts, Design, Literature search, Data analysis, Statistical analysis, Manuscript preparation.*

M. Raffaq Saleem; PGR: *Literature search, Data acquisition, Manuscript review.*

Fuad Ahmad Khan Niazi; Professor: *Manuscript preparation, Manuscript editing, Manuscript review.*