Evaluation and comparison of conjunctival swab polymerase chain reaction results in SARS-CoV-2 patients with and without ocular manifestations

Prempal Kaur, Gaurang Sehgal, Shailpreet, KD Singh, Bhavkaran Singh

Purpose: The aim of this study was to evaluate and compare the presence of SARS-CoV-2 in tears of patients with and without ocular symptoms in SARS-CoV-2 positive patients. Methods: The prospective observational study conducted on 60 consecutive SARS-CoV-2 positive patients with ocular complaints was compared with 60 controls who had no ocular manifestations. The tear samples were taken within 48 h of admission from both the eyes of the enrolled patients for evaluating the presence SARS-CoV-2 by reverse transcription-polymerase chain reaction. Results: Eleven cases (18.33%) tested positive for SARS-CoV-2 in tears on RT-PCR from conjunctival swab compared to 10 (16.66%) controls. The difference was not statistical significant. The difference between mean age of patients who tested positive or negative was also without statistical significance (P = 0.652), but the difference between patients who tested positive or negative by conjunctival swab for SARS-CoV-2 was statistically significant in terms of severity of COVID-19 disease (P = 0.0011), presence of comorbidity (P = 0.0015), mean TLC (P = 0.00498), and mean dimer (P = 0.00465). Conclusion: Though the percentage of patients with positive RT PCR from conjunctival secretions is significantly less than nasopharyngeal swabs, potential risk of transmission of SARS-CoV-2 through tears cannot be ruled out. Moreover, SARS-CoV-2 can be present in tears irrespective of ocular involvement.

Key words: Conjunctival secretions, ocular manifestations, RT PCR, SAR, S-CoV-2

On March 11, 2020, World Health Organization declared coronavirus as a global pandemic.[1] Although the commonest presentation of COVID-19 at the outset includes respiratory symptoms with fever, myalgia, fatigue, and diarrhea,[2] the frequency of ocular symptoms are not uncommon in COVID-19.[3] Since ocular surface could serve as a potential port of entry and ocular secretions as possible reservoir and route of transmission of SARS-CoV-2 is under discussion, the objective of this study was to evaluate and compare the presence of SARS-CoV-2 in tears of patients with and without ocular symptoms in SARS-CoV-2 positive patients.

Methods

After taking clearance from the institutional ethical committee, this prospective interventional study was conducted in Department of Ophthalmology in collaboration with Viral research and diagnostic lab on laboratory confirmed (with a real-time RT-PCR assay of oropharyngeal and nasopharyngeal swab) novel coronavirus patients admitted in medical wards. One hundred twenty patients of SARS-CoV-2 were enrolled and divided equally into two groups, 60 with (Group A) and 60 without ocular manifestations (Group B). Written informed consent in vernacular language was taken from enrolled patients willing to participate in the study in advance in accordance with the Declaration of Helsinki. Patients having preexisting ocular complaints and very severe cases who were intubated were excluded from the study.

Moderate cases, defined as those having clinical signs of pneumonia and with oxygen saturation (SpO2) measured by pulse oximetry <94% (90–95%) on room air and respiratory rate ≥24/min and severe cases, identified when with clinical signs of pneumonia patient had one of the following sign: Respiratory rate >30 breaths/min; severe respiratory distress or SpO2 <90% on room air were included.

After recording brief history and demographic profile of the enrolled patients, symptoms, ocular findings including ocular surface, anterior segment assessment was performed by the same experienced clinician and recorded. Results of CT scan (Chest), blood tests, and RT PCR from nasopharyngeal and oropharyngeal swabs were noted.

The tear samples were taken within 48 h of admission from both the eyes of the patients using conjunctival swab and Schirmer paper strips. For taking the conjunctival swab, lower eyelid was retracted and inferior fornix was swept with sterile disposable nylon swab for 10 s and the procedure was repeated in the second eye. In addition, the sample was taken using Schirmer
paper strip (no 41 Whatman filter paper, 5 mm wide and 35 mm long) without putting topical anesthesia. It was folded at one end and placed at the junction of middle third of the lower lid of both eyes. The patient was asked to keep the eye open and blink normally, and after 3 min, the strips were removed. All the four samples were then placed in a single viral transport medium which after proper labeling and sealing and while maintaining temperature of 4°C was transferred to VDRL Lab in a triple layer packing for evaluating the presence of SARS-CoV-2 by reverse transcription-polymerase chain reaction. Multiplex PCR was used by using kits approved by ICMR. Reporting (positive/negative) was done by following manufacturer’s guidelines. Both screening (E gene) as well as confirmatory (orf/rdrp/N gene) assays were done on each sample.

Statistical analysis

Data was statistically analyzed using SPSS version 23 (Armonk, NY: IBM Corp.) Data was summarized using range, mean ± standard deviation, median, and percentiles for quantitative variables or frequency and percentage for qualitative ones. Comparison between groups was performed using Mann–Whitney U-test for quantitative variables and Chi-square or Fischer’s exact test for qualitative variables. A P value of < 0.050 was considered statistical significant.

Results

A total of 60 SARS-CoV-2 positive patients with ocular manifestations (Group A) and 60 without ocular manifestations (Group B) were enrolled in the study. Mean age of Group A and Group B patients was 54.5 ± 1.68 and 56.35 ± 1.90, respectively. Male: Female ratio of Group A and Group B was 2.52:1 and 2:1, respectively. Twenty-two patients (36.6%) of Group A had moderate COVID-19 disease, while 38 patients (63.3%) had severe infection, whereas 31 patients (51.66%) of Group B had moderate disease and 29 (48.33%) had severe disease. Ocular manifestations included conjunctival hyperemia in 41 patients (68.3%), follicular reaction in 38 patients (63.3%), chemosis in 35 patients (58.3%), mucoid discharge in 20 patients (33.3%), and itching in 11 (18.3%). Six (1%) patients had conjunctivitis as the initial symptom, even before the onset of fever, malaise, or pulmonary symptoms. The difference between values of D-dimer and total leucocyte count in Group A and B was statistically significant. Among 60 Group A patients, 11 (18.33%) tested positive for SARS-CoV-2 in tears on RT-PCR from conjunctival swab compared to 10 (16.66%) Group B patients without ocular manifestion. The difference between two groups was not statistical significant [Table 1].

In total, 17.5% of patients (21 of 120) under evaluation for RT PCR of tears were positive for SARS-CoV-2 out of which 11 patients (9.16%) had ocular manifestations and 10 (8.33%) did not have any ocular complaint. The difference between them was not statistically significant. The difference between mean age of patients who tested positive or negative was also without statistical significance (P = 0.652), but the difference between patients who tested positive or negative by conjunctival swab for SARS-CoV-2 was statistically significant in terms of severity of COVID-19 disease (P = 0.0011), presence of comorbidty (P = 0.0015), mean TLC (P = 0.00498), and mean d dimer (P = 0.00465) [Table 2].

Table 1: Demographic profile of COVID-19 patients under evaluation

|                      | Group A (60) | Group B (60) | P       |
|----------------------|--------------|--------------|---------|
| Mean age             | 54.5±1.68    | 56.35±1.90   | <0.001  |
| Sex                  |              |              |         |
| Males                | 43           | 40           | 0.553   |
| Females              | 17           | 20           |         |
| Mean duration of disease | 2.66±0.18   | 2.68±0.19    | 0.555   |
| Severity of disease  |              |              |         |
| Moderate             | 22 (36.6%)   | 31 (51.66%)  | 0.090   |
| Severe               | 38 (63.33%)  | 29 (24.9%)   |         |
| Mean total leucocyte count | 12138±986  | 9965±890    | <0.001  |
| Mean D dimer         | 0.925±0.160  | 0.698±0.118  | <0.001  |
| RT PCR               | 11           | 10           | 0.810   |

When comparing the mean values, t-test was used otherwise Chi-square test

Table 2: Comparison of conjunctival swab PCR results in SARS-CoV-2 patients with various variables

| Ocular manifestation | Positive RT PCR | Negative PCR | P       |
|----------------------|-----------------|--------------|---------|
| Yes                  | 11              | 49           | 0.810   |
| No                   | 10              | 50           |         |
| Mean age             | 52.66±0.9       | 56.35±1.6    | <0.001  |
| Severity of disease  |                 |              |         |
| Moderate             | 7 (33.3%)       | 46 (46.4%)   | 0.390   |
| Severe               | 14 (67.6%)      | 53 (53.6%)   |         |
| Comorbidty           |                 |              |         |
| Yes                  | 19              | 81           | 0.334   |
| No                   | 2               | 18           |         |
| Mean TLC             | 13499±223       | 9777±165     | <0.001  |
| Mean d dimer         | 0.96±0.215      | 0.68±0.109   | <0.001  |

When comparing the mean values, t-test was used otherwise Chi-square test

Discussion

The primary transmission of COVID-19 is via respiratory droplets; however, risk of transmission via other routes such as fecal oral and conjunctival secretions cannot be ignored. In fact Li Wang, an ophthalmologist, who later died from COVID-19 was the first to voice concern regarding spread of COVID-19. He was believed to have contacted virus from asymptomatic glaucoma patient.[4] Ocular manifestations in COVID-19 patients vary between 0.8 and 31.6%,[5] Xia et al. reported that the only patient with conjunctivitis out of 30 hospitalized COVID-19 patients tested positive for SARS-CoV-2 in ocular secretions.[6] However, Sun et al.[7] concluded that the eye is rarely involved in human CoVs infection. On the contrary, some clinicians have expressed concerns about the transmission of SARS-CoV-2 via tears and conjunctival secretions of infected patients.[8] The American Academy of Ophthalmology in view of possible transmission, in its recent publication, has also
advised all contact lens wearers to switch to glasses during this pandemic. A recent report also raised doubts when one-third of eye professionals accidentally acquired COVID-19 as severe as resulting in three deaths while managing patients during this pandemic. Also another study concluded that out of two patients who reported conjunctivitis, one tested positive via RT-PCR from conjunctival swab. She was a 29-year-old nurse and while working in the emergency department at Tongji hospital, Wuhan city, China, she continuously wore N-95 respirator but often removed her goggles and touched her eye lids. Nonetheless, polymerase chain reaction on tears from patients with SARS-CoV infection has also demonstrated presence of virus even in the absence of conjunctivitis. Research into establishing the presence of COVID-19 virus in conjunctival secretions would be valuable in developing preventive strategies.

Confirming with Hany Mahmoud et al. who detected SARS-CoV-2 in 28.57% (8 out of 28) patients, our research also demonstrated SARS-CoV-2 in tears and conjunctival secretions of 17.5% (21 out of 120) patients. Unlike our observations, Mahmut Atum et al. reported a positivity rate of 7.5% in 40 patients, Wu et al. 5.2% in 28 patients, Zhang et al. 1.3% in 72 patients, and Xia et al. 6.6% in patients for SARS-CoV-2 using conjunctival swab RT-PCR. Low incidence of positive conjunctival swab in COVID-19 patients in these studies may be accounted to low sample size or insufficient tear material to detect the virus. We tried to increase the quantity of sample of conjunctival secretions first by taking the sample simultaneously from both the eyes and second by using schirmer strips in addition to conjunctival swab. Moreover, the viral load is known to fall in the second and third week of symptoms. To overcome this, we took the sample within 48 h of onset of ocular complaints when the viral load seems to be higher.

Of 21 patients who tested positive for SARS-CoV-2, 11 (9.16%) patients had ocular manifestations and 10 (8.33%) did not have any ocular complaint. The difference was not statistically significant suggesting that COVID-19 patients can shed SARS-CoV-2 in conjunctival secretions even in the absence of ocular involvement. It was similar to the observations by Hany Mahmoud et al. in Egypt, where out of 10 patients with conjunctival findings only three patients had SARS-CoV-2 in their conjunctival secretions using RT-PCR test and the remaining five patients with virus in their conjunctiva did not have any ocular complaint. Mahmut Atum et al. also confirmed that the difference between patients who tested positive or negative for SARS-CoV-2 using conjunctival swab was without statistical significance in terms of the presence of conjunctivitis \( P = 0.720 \). Moreover, 5 of 32 patients (16%) without conjunctivitis were also reported to have viral RNA in their tear-conjunctival samples. In contrast to the observations of Xia et al. who in their prospective interventional case series on 30 patients with confirmed novel coronavirus pneumonia demonstrated that SARS-CoV-2 was present in the tears and conjunctival secretions of patients with conjunctivitis only and no virus was detected in the tears or conjunctival secretions of patients without conjunctivitis.

Meanwhile, a study from Italy observed ocular manifestations in 26.2% of their hospitalized patients but conjunctival swabs of none of them tested positive for SARS-CoV-2. Further

Seah IYJ et al. in their study assessed SARS-CoV-2 by viral isolation and RT-PCR in 17 COVID-19 patients, and all samples showed negative results for SARS-CoV-2.

A comparison of D-dimer and total leucocyte count was made between patients who tested positive or negative on conjunctival swab RT-PCR results, and our results showing raised D-dimer and total leucocyte count in patients with positive swab were statistically significant. It was similar to the reports of Wu et al. but Mahmut Atum et al. did not find it to have statistical significance.

In addition to laboratory findings, correlation of positivity of conjunctival swab for SARS-CoV-2 and severity of disease was investigated in the current study. In total, 71.4% patients (15) with positive RT-PCR were reported to be having severe disease. Wu et al. also revealed that both his patients with positive RT-PCR were critical COVID-19 patients. Another study reported that the two patients who tested positive for SARS-CoV-2 in conjunctival specimen were elderly people with severe forms of the disease.

All these observations though variable suggest that tears can be a potential source of infection for health care workers. Ophthalmologist need to be more cautious because of close proximity to patient’s nose, mouth, and tears while examining the patient. In wake of published reports that RNA shedding can occur even in asymptomatic patients, it becomes all the more important for ophthalmologists not to examine any patient during pandemic with unprotected eyes. It is recommended that all health care professionals in addition to strict hand hygiene should wear face masks and protective glasses while examining patients during COVID-19 pandemic.

Limitations

Our study had several limitations. First, small sample size and that also from a single medical center. Second, we excluded the patients with previous history of ocular symptoms like itching, watering, or refractive disorder. If we had taken that in account, repeated touching of eyes might have explained tears as one of the route of transmission. Third, we detected viral shedding while doing RT-PCR from conjunctival secretions. It would have been more reliable to confirm the transmission route if could isolate live virus. Finally, we took sample only once. Multiple samples at variable time would have helped establish the duration of infectivity of tears.

Conclusion

Though the percentage of patients with positive RT PCR from conjunctival secretions is quite less than those with positive nasopharyngeal swabs, potential risk of transmission of SARS-CoV-2 through tears still cannot be ruled out. Moreover, SARS-CoV-2 can be present in tears with or without ocular involvement; therefore, prevention is the most important aspect to be remembered by all emergency physicians and ophthalmologists to protect their patients and themselves by adopting required precautions.

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Conflicts of interest

There are no conflicts of interest.
References

1. World Health Organization. World Health Organization Coronavirus Disease 2019 (COVID-19) Situation Report. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.

2. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 2020;109:102433.

3. Wu P, Duan F, Luo C, Liu Q, Qu X, Liang L, et al. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol 2020;138:575-8.

4. Hu K, Patel J, Swiston C, Patel BC. Ophthalmic manifestations of coronavirus (COVID-19). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.

5. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.

6. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol 2020;92:589-94.

7. Sun CB, Wang YY, Liu GH, Liu Z. Role of the eye in transmitting human coronavirus: What we know and what we do not know. Front Public Health 2020;8:155.

8. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet 2020;395:65.

9. Mukamal R, Tuli SS. Coronavirus Eye Safety. American Academy of Ophthalmology (AAO); 2020. https://www.aao.org/eye-health/tips-prevention/coronavirus-covid19-eye-infection-pinkeye.

10. Qiao C, Zhang H, He M, Ying G, Chen C, Song Y, et al. Symptomatic COVID-19 in eye professionals in Wuhan, China. Ophthalmology 2020;127:1268-70.

11. Zhang X, Chen X, Chen L, Deng C, Zou X, Liu W, et al. The evidence of SARS-CoV-2 infection on ocular surface. Ocul Surf 2020;18:360-2.

12. Loon SC, Teoh SC, Oon LL, Se-Thoe SY, Ling AE, Leo YS, et al. The severe acute respiratory syndrome coronavirus in tears. Br J Ophthalmol 2004;88:861-3.

13. Kaya H, Çalışkan A, Okul M, San T, Akbudak IH. Detection of SARS-CoV-2 in the tears and conjunctival secretions of Coronavirus disease 2019 patients. J Infect Dev Ctries 2020;14:977-81.

14. Mahmoud H, Ammar H, El Rashidy A, Ali AH, Hefny HM, Mounir A. Assessment of Coronavirus in the conjunctival tears and secretions in patients with SARS-CoV-2 infection in Sohag Province, Egypt. Clin Ophthalmol 2020;14:2701-8.

15. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177-9.

16. Atum M, Boz AA, Çakır B, Karabay O, Köroğlu M, Öğütli A, et al. Evaluation of conjunctival swab PCR results in patients with SARS-CoV-2 infection. Ocul Immunol Inflamm 2020;28:745-8.

17. Cavalleri M, Brambati M, Starace V, Capone L, Nadin F, Pederzoli M, et al. Ocular features and associated systemic findings in SARS-CoV-2 infection. Ocul Immunol Inflamm 2020;28:916-21.

18. Seah FY, Anderson DE, Kang AE, Wang L, Rao P, Young BE, et al. Assessing viral shedding and infectivity of tears in coronavirus disease 2019 (COVID-19) patients. Ophthalmology 2020;127:977-9.

19. Güemes-Villahoz N, Burgos-Blasco B, Arribi-Vilela A, Arriola-Villalobos P, Rico-Luna CM, Cuiña-Sardiña R, et al. Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? J Med Virol 2021;93:383-8.