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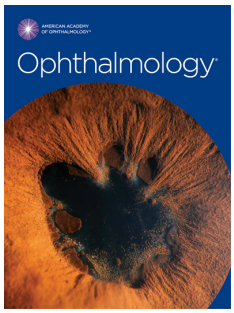

Assessing Viral Shedding and Infectivity of Tears in Coronavirus Disease 2019 (COVID-19) Patients

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Title Page

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Abstract

Ocular transmission of COVID-19 is uncertain. 64 tear samples were collected from 17 COVID-19 patients between Day 3 to Day 20 from initial symptoms. Neither viral culture nor reverse transcription polymerase chain reaction (RT-PCR) detected the virus, suggesting a low risk of ocular transmission.

Main Manuscript

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has rapidly spread across the globe to cause a pandemic. While it is known to be transmitted via droplets, alternative modes of transmission remain unknown. Transmission through infected ocular tissue or fluid has been a controversy[1,2]. It is hypothesized that the nasolacrimal system can act as a conduit for viruses to travel from the upper respiratory tract to the eye. Hence, ocular tissue and fluid may represent a potential source of SARS-CoV-2. In this study, we attempted to determine the possibility of transmission through tears by assessing for the presence of SARS-CoV-2 with viral isolation and quantitative reverse transcription polymerase chain reaction (RT-PCR). As patients were being monitored clinically via routine nasopharyngeal swabs (NP), they were compared with tears to further understand patterns of viral shedding.

17 COVID-19 patients were recruited for this prospective study in Singapore after obtaining informed consent. This study was carried out in accord with the Declaration of Helsinki and ethics approved by the Domain Specific Review Board of the National Healthcare Group (NHG) Singapore. NPs were collected routinely for clinical monitoring of patient's condition while tear samples were collected purely for research purposes. On some days, both tears and NPs were collected at the same time. These samples were delivered to different labs for processing.

COVID-19 patients were tested positive by RT-PCR of NPs in a clinical diagnostic laboratory. NPs were collected in universal viral transport media and RNA extraction done using NucliSENS® easyMAG® system (bioMérieux). 55µl of the elute was then used to perform RT-PCR as per manufacturer's instructions using the A*STAR FORTITUDE kit (Accelerate

Technologies Pte .Ltd, Singapore). The limit of detection was estimated to be <25copies of RNA.

Tears were sampled by a senior consultant ophthalmologist using Schirmer's test strip at varying timepoints between Day 3 and 20 after the initial development of symptoms. Caution was taken to prevent contamination of samples. The Schirmer's strip tear collection method was previously validated in other studies[3]. Samples from both eyes were taken and analysed separately. Collected strips were placed into individual falcon tubes of universal viral transport media. Samples were delivered to a research laboratory for processing. Samples were used to inoculate Vero-E6 cells (ATCC®CRL-1586TM). After 4 days of incubation, cells were observed for the presence of cytopathic effect (CPE). Total RNA was extracted from all samples using E.Z.N.A. Total RNA Kit I (Omega Bio-tek) according to the manufacturer's instructions and samples were analysed by real-time quantitative reverse transcription-PCR (RT-qPCR) for the detection of SARS-CoV-2 as previously described[4].

Clinical data including age, sex, symptoms, nasopharyngeal swab results were collected from electronic health records and correlated with RT-PCR results. Ocular symptoms which were assessed include red eye, tearing, blurring of vision, discharge and colour desaturation. These symptoms were chosen based on the ocular manifestations of other coronaviruses known to infect humans and animals[2]. Other symptoms of COVID-19 assessed include fever, cough, shortness of breath, rhinorrhea and sore throat.

Of the 17 patients recruited, none presented with ocular symptoms. However, 1 patient developed conjunctival injection and chemosis during the stay in the hospital **(Table 1 available at www.aaojournal.org).** 14 patients presented with upper respiratory tract symptoms including cough, rhinorrhea and sore throat.

A total of 64 samples were taken over the study period, with 12, 28 and 24 samples taken from first, second and third week of initial symptoms respectively. All were tested negative for the SARS-CoV-2 on viral isolation and RT-PCR. Tear results were compared with NP results as shown in **Figure 1**. Ct values of NP swabs were featured.

To our knowledge, this is the first study comparing viral shedding in tears with NP results during the course of COVID-19 infection. A previous study showed positive SARS-CoV-2 RT-PCR results from a patient's tears, but isolation of the virus was unsuccessful[5]. In this study, there was no evidence of SARS-CoV-2 shedding in tears through the course of the disease. Viral load detected in nasal and throat swabs are elevated for a period of approximately 2 weeks from the onset of COVID-19 symptoms[6]. In this study, the tear sampling timepoints cover these 2 weeks of active infection, providing a good representation of the full disease course. All tear samples tested negative even when NPs continued to test positive. Furthermore, patients with symptoms of upper respiratory tract infections did not demonstrate any viral shedding in tears, suggesting the hypothesis of the lacrimal duct as a viral conduit may not be true. Most importantly, only one patient developed ocular symptoms during the disease course and no evidence of SARS-CoV-2 could be found in the tear samples. This suggests that transmission through tears regardless of the phase of infection is likely to be low.

The study had several limitations. Firstly, the samples were analysed in different laboratories utilising two different assays. As the NPs were utilised in the clinical setting to monitor disease progression, they were analysed in a clinical diagnostics lab while the tear samples were analysed in a research lab. While the limit of detection for the research lab was not assessed due to logistical limitations, it should be noted that the tear samples were

incubated with Vero-E6 cells for 4 days prior to obtaining the RNA for RT-PCR. If SARS-CoV-2 existed in the samples, CPE would have been observed even in a false negative RT-PCR result. We observed neither CPE nor a positive RT-PCR result, thereby the likelihood of SARS-CoV-2 being found in the tear samples is still low. Secondly, only tears were sampled rather than conjunctival tissue. In the pandemic setting, COVID-19 patients are already emotionally distraught with their diagnosis. Hence, conjunctival tissue sampling was avoided to reduce patient distress. Despite this, we believe that our results do highlight a low risk of ocular transmission. In the acute infection of conjunctival cells, cells die through viral-mediated lysis or from immune reactions. Cell death will release viral material into tears which can still be detected via RT-PCR. Thirdly, the study had a small sample size due to the logistical limitations of the outbreak response. These patients also usually present a few days after symptom development, making sampling during early infection difficult. Finally, only 1 patient had ocular symptoms in our study. However, studying patients with ocular symptoms can be difficult. In a study of 1099 COVID-19 patients, only 0.8% developed conjunctival congestion[7].

The results from this study suggests that the risk of SARS-CoV-2 transmission through tears is low. However, further definitive mechanistic studies are required. SARS-CoV-2 has been known to infect cells via ACE2 receptors. More studies are required to definitely prove the presence of ACE2 on corneal and conjunctival cells. Future studies involving more patients with ocular symptoms should also be considered. Finally, future studies should consider the association between serum viral load and viral shedding in tears. Unfortunately, no blood samples were analysed for this experiment as they were not routine clinical investigation in the management of patients.

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Figure Legends

Figure 1: Comparison of Tear Samples and Nasopharyngeal Sawb Samples Over Course of COVID-19 Illness

CT results of all nasopharyngeal swabs are displayed. All tear samples were tested neagtive for on both viral isolation & RT-PCR. These results were labelled by a red coloured box.

Patient Serial Number	Days Since Initial COVID-19 Symptoms																			Discharge Status	Total Duration of Symptoms (As of 12/3/20)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	
1		24.4	20.11	19.09	NA	21.65	24.00	23.46	23.17												Still admitted	6 Days
2		24.3	37.92	NA	NA	32.75	-	-													D9 Discharge	5 Days
3			27.06	22.28	NA	22.41	24.30	28.50	30.31	26.67	25.80	27.99	38.05	-							Still admitted	5 Days
4					21.20	22.04	NA	NA	24.51	28.4	28.24	NA	30.54	NA	34.86	NA	36.83	NA	37.17	NA	Still admitted	18 Days
5					29.48	26.19	NA	day 8	28.69	NA	NA	NA	NA	34.07	NA	NA	- *	35.48 *	NA *	NA *	Still admitted	Still Symptomatic (23 Days)
6	33.5	-	31.18	-	36.28	-	-														D8 Discharge	2 Days
7						37.70	NA	35.02	NA	34.69	35.09	NA	-	-							D15 Discharge	11 Days
8								26.33	NA	NA	NA	NA	29.15	NA	37.05	NA	37.1	NA	35.35	NA	Still admitted	18 Days
9								31.22	33.71	NA	34.17	NA	NA	34.63	NA	NA	-	34.25	29.04	35.33	Still admitted	11 Days
10						-	-	-	NA	34.10	-	-									D14 Discharge	12 Days
11								29.19	NA	NA	33.72	36.20	NA	36.71	NA	NA	33.13	NA	35.14	NA	Still admitted	15 Days
12								37.55	32.79	33.43	38.16	32.28	39.39	35.91	37.72	38.42	38.21	-	38.21	36	D25 Discharge	23 Days
13											29.54	NA	NA	33.04	NA	NA	NA	-	37.55	NA	Still admitted	22 Days
14										29.30	34.00	33.31	28.66	35.27	34.53	30.77	37.45	35.92	32.29	-	Still admitted	6 Days
15						25.40	NA	NA	NA	26.38	33.06	32.26	30.20	36.21	29.48	-	-				D17 Discharge	11 Days
16				22.89	NA	22.15	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	38.81	-	36.34	-	D21 Discharge	20 Days
17					19.35	NA	20.01	NA	21.97	29.06	32.19	32.27	21.31	19.22	34.10	32.05	30.43	-	36.52	-	D22 Discharge	15 Days

Legend	
-	Negative Nasopharyngeal Swab
NA	No Nasophayrngeal Swab Taken
*	Ocular Symptoms
	Tears Sampled Negative
	Tears Sampled Positive

Figure 1: Comparison of Tears Samples and Nasopharyngeal Swab Samples Ct Values Over Course of COVID-19 Illness