Role of Reverse Transcriptase Polymerase Chain Reaction in Cornea Donors During the COVID-19 Pandemic

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Purpose: The purpose of this study was to report the analysis of reverse transcriptase polymerase chain reaction (RT-PCR) of nasopharyngeal (NP) samples of cornea donors dying because of causes unrelated to severe acute respiratory coronavirus (SARS-CoV-2).

Methods: A retrospective analysis of all cornea donors dying from causes other than SARS-CoV-2 between August 2020 and December 2020 was performed. Informed consent was obtained from the next of kin of the deceased for RT-PCR testing from NP swabs. Rapid antigen testing from all the deceased was performed before in situ cornea excision. In addition, NP samples in viral transport media for RT-PCR were also collected for SARS-CoV-2 analysis. Corneas were released from the eye bank only after a negative RT-PCR report.

Result: One hundred eighteen corneas from 59 donors were obtained by the eye bank. Eleven donors (18.64%) were positive for SARS-CoV-2 on RT-PCR testing. Six of these 11 donors had a Ct value of E gene less than 25.

Conclusions: NP samples of cornea donors dying due to causes other than coronavirus disease-19 were positive for SARS-CoV-2 on RT-PCR. This implicates that donors could be having asymptomatic/undetected coronavirus disease infection. We recommend adding the routine testing of NP samples of all cornea donors in the eye banking protocol in this ongoing SARS-CoV-2 pandemic.

Key Words: SARS-CoV-2, cornea transplantation, eye banking, COVID-19

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The coronavirus disease (COVID)-19 pandemic has led to a drastic fall in eye banking activities for donor cornea retrieval globally. With many uncertainties and the potential of severe acute respiratory coronavirus (SARS-CoV-2) to affect the ocular surface, eye banking around the world had serious challenges.

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SARS-CoV-2 has been isolated in conjunctival swabs and tears of patients with COVID-19 with ocular route acting as both portal of entry and carrier of the virus.[1] The route of transmission and infiltration of the virus within the ocular tissue is still unknown. There is, however, lack of evidence that SARS-CoV-2 can be transmitted through corneal transplantation. Also, to date, there have been no reported cases of recipient-donor SARS-CoV-2 transmission through human cell or tissue. Conflicting reports of viral RNA in the cornea of the patients dying from COVID-19 exist.[2–4] With the risk of transmission of a potentially fatal systemic disease through corneal transplantation being not acceptable, eye banking guidelines preclude retrieval of tissues from donors recently infected with or exposed to COVID-19.

A report from eye banks of 26 European countries emphasizes the consensus recommendations for donor cornea screening to take into considerations polymerase chain reaction (PCR) on nasopharyngeal swabs (NP swabs) at the time of cornea procurement from potential donors with a history of respiratory symptoms compatible with COVID-19.[5] However, there is no consensus on routine COVID testing in NP swabs of deceased donors with no known exposure to COVID-19. Thus, the challenge of screening donors dying from causes other than COVID-19, with the possibility of them being asymptomatic carriers for COVID-19 remains.

A preliminary protocol was designed internally in an eye bank at New Delhi in India after the resumption of eye banking activities and gradual lifting of restrictions in August 2020 to test the NP swabs of all the cornea donors during this ongoing pandemic. The eye bank association of India recommended collection of nasal swabs of deceased donors for reverse transcriptase PCR (RT-PCR) testing for COVID-19 at the discretion of the hospital director. Eye banks were also suggested to exclude cornea retrieval from confirmed or suspected COVID-19 donors or those who had conjunctivitis or pneumonia.[6] Because the approval of RT-PCR testing was under the discretion of the eye bank director, we added the mandatory testing of nasal swabs for COVID-19 of donors in to the protocol before releasing donated corneas for transplantation. Corneas were released for use only after negative real-time RT-PCR on the nasopharyngeal swab for COVID-19 was received by the eye bank.

Analysis of RT-PCR of nasopharyngeal swabs of cornea donors who had died due to causes other than COVID and had no history of respiratory symptoms compatible with COVID-19 was performed. The cycle threshold (Ct) values of E gene and RdRp gene were also assessed to estimate the viral load.

MATERIALS AND METHODS

A retrospective analysis of RT-PCR of all the cornea donors between August 2020 and December 2020 was performed. In situ cornea excisions were performed in 59 donors after ensuring that NP swabs of the deceased were negative on rapid antigen testing [STANDARD Q COVID-19 Ag (SD-Biosensor, RELAB, I); sensitivity range 62.8%–86.4%] for SARS-CoV-2. Informed consent from the next of kin for conducting RT-PCR from nasopharyngeal samples of the deceased was obtained before cornea retrieval, as part of the eye bank protocol. Voluntary donations obtained from both the mortuary and the hospital were included in this study. None of the donors had known cases of COVID-19 or had close contact with patients with SARS-CoV-2 within 15 days of their death. They were asymptomatic for COVID and not suffering from any respiratory insufficiency or pneumonia. In situ excision was performed by an eye bank technician donning personal protective equipment to avoid the risk of contracting COVID. All precautions were taken to prevent inadvertent transmission of COVID infection to the personnel while collecting, transporting, and processing the donor eyes. Another nasopharyngeal swab sample from the donor was also collected before in situ excision in viral transport media for RT-PCR testing. The donor corneas were stored in Cornisol medium (Aurolab, India) after retrieval. Before in situ excisions, double disinfection with povidone-iodine for 4 minutes each was performed on the ocular surface. The eye bank follows a specific protocol as a precautionary measure to limit the transmission of COVID-19 to eye bank personnel and further to the recipient: hand hygiene, personal protective equipment, disposable materials, draping and sterile preparation of the recovery area, and pathogen reduction with povidone-iodine being some of them. The nasopharyngeal swab samples were stored at 4°C for 6 to 24 hours in viral transport media and transported, maintaining the cold chain to the microbiology department. The eye bank and microbiology department are part of the teaching facility managed by the state government, Delhi, India. The RT-PCR was performed to test the presence of SARS-CoV-2 RNA of the sarbecovirus envelope gene (E gene) per the guidelines of the National Institute of Virology.[7] The viral load from the swabs was also assessed for the Ct value of the E gene and the RdRp gene. Primer sequences used for amplification of the E gene and the RdRp gene are given in Table 1.[8]

A specimen was considered to have confirmed positive results for the 2019 novel coronavirus if the reaction growth curves crossed the threshold line within 35 cycles for the E gene and the RdRp gene. Viral loads from samples were classified as high (Ct values < 25), medium (Ct values 25–30), and low (Ct values > 30).[7] The cost for RT-PCR testing is borne by the state government, amounting to US $ 12, and results are made available within 24 hours of the sample collection at the microbiology department.

RESULTS

A total of 118 corneas from 59 donors were obtained from August 2020 to December 2020. Among these, 80 corneas were collected through home donations and 38 corneas from the mortuary/hospital. The age range of donors was 17 to 93 years. NP swabs from 11 donors (18.64%) were positive for SARS-CoV-2 on RT-PCR testing. For 2 of these donors, the cause of death was hanging and the corneas were retrieved during postmortem. For the other 9 donors, 7 died at home and 2 died at the hospital from a cerebrovascular accident. One of the donors (case 11) that died at the hospital had a negative RT-PCR nasopharyngeal swab before death, whereas the RT-PCR repeated at cornea donation was reported to be positive. The case positivity rate of SARS-CoV-2 in Delhi (India) during the period of this study ranged from a maximum of 14% to a minimum of 1.8%.[9]

Ct values for the E gene in the 11 donors with positive RT-PCR ranged between 14 and 34. There were 6 donors (54.54%) with Ct values less than 25, 2 donors (18.18%) with Ct values between 26 and 30, and 3 donors (27.27%) with Ct values between 31 and 34. Ct values less than 25 in 54.54% of COVID-19–positive donors suggested a high probability of COVID-19 transmission. The cause of death, type of donation, and Ct values of the cornea donors are provided in Table 2. None of them had any reported respiratory symptoms related to COVID-19. All the corneas retrieved from these donors were not released for corneal transplantation.

DISCUSSION

There exists a risk of transmitting infectious diseases and viruses through corneal transplantation, which can affect recipients or those handling donor tissues.[10] Emergence of the novel SARS-CoV-2 in December 2019 has affected corneal transplantation worldwide with a significant decrease in the donor pool and differences between countries regarding donor screening algorithms based on precautionary principles. There is definite consensus currently on excluding

TABLE 1. Primers and Probes for the Indian Council of Medical Research—National Institute of Virology E Gene and RdRp Gene

Assay/Use	Oligonucleotide ID	Sequence (5′–3′)
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_R27	ATATTGCAGCAGTACGCACACA
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ
RdRp gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA
	RdRP_SARSr-P2 specific for Wuhan-CoV	FAM-CAGGTGGAACCTCATCAGGAGATGCQSY

TABLE 2. Age, Cause of Death, and Ct Values of Cornea Donors Dying Because of Causes Unrelated to SARS-CoV-2

S. No	Age	Sex	Cause of Death	Type of Donation	Ct Value (E Gene) of Nasopharyngeal Swab	Ct Value (RdRp Gene) of Nasopharyngeal Swab
1	17 yr	M	Hanging	Mortuary	16	16
2	67 yr	F	Natural	Voluntary	14	13
3	57 yr	F	Stroke	Voluntary	14	14
4	81 yr	M	Natural	Voluntary	26	26
5	85 yr	M	Typhoid	Voluntary	20	19
6	19 yr	F	Hanging	Mortuary	28	29
7	74 yr	M	Natural	Voluntary	33	33
8	79 yr	M	Natural	Voluntary	23	24
9	25 yr	M	Cardiac arrest	Mortuary	33	32
10	93 yr	M	Cardiac arrest	Voluntary	34	34
11	74 yr	M	CVA	Voluntary	23	26

donors with confirmed COVID-19 for the fear of transmitting COVID to the recipient through corneal transplantation.[11]

SARS-CoV-2 has been found in conjunctival swabs and tears of patients with COVID-19.[1] Arora et al reported SARS-CoV-2 RNA in tears of 24% of patients with laboratory-proven moderate to severe COVID-19.[7] However, Seah et al[12] showed negative results for viral RNA from all the tear samples evaluated, even when the nasopharyngeal swab samples continued to show positive results. Casagrande et al[13] detected the presence of SARS-CoV-2 RNA in the retina of deceased patients with COVID-19. Postmortem eyes and surgical specimens have been analyzed for the expression of ACE2 (the receptor for SARS-CoV-2) and TMPRSS2, a cell surface–associated protease that modulates viral entry after binding of the viral spike protein to ACE2.[14,15] The presence of ACE2, TMPRSS2, and DCSIGN/DC-SIGNR in corneal epithelium and endothelium indicates that multiple layers of the cornea may be susceptible to infection by the virus.

Currently, there are conflicting reports on the presence of SARS-CoV-2 RNA in ocular tissues from COVID-19 donors. Bayyod et al[3] and List et al[4] reported no RNA detection on qRT-PCR. Sawant et al[2] reported 3 conjunctival, 1 anterior corneal, 5 posterior corneal, and 3 vitreous swabs to be positive for SARS-CoV-2 RNA from the samples taken from the 20 eyes recovered from 10 donors with COVID-19. They have recommended that in view of small but noteworthy prevalence of SARS-CoV-2 in ocular tissues of COVID-19 donors, a postmortem nasopharyngeal PCR testing protocol should be followed to eliminate the use of any tissue potentially harboring SARS-CoV-2 for cornea transplantation. Recently, Casagrande et al[16] reported the presence of viral genomic and subgenomic RNA of SARS-CoV-2 in the cornea of patients with COVID-19 viremia. Low RNA loads in cornea samples suggest a low risk of infection through a corneal transplant, even in a high-risk cohort of patients with viremia. They concluded that further research is warranted to assess the rate of SARS-CoV-2 transmission because infection through a contaminated corneal graft cannot be fully excluded.

The recommended diagnostic method for potential donor screening of SARS-CoV-2 infection is viral RNA detection in NP swabs specimen on RT-PCR.[17] Upper respiratory tract swabs in the form of NP swabs is the recommended method for COVID testing from postmortem swab specimens of the deceased of confirmed or suspected COVID-19.[18] However, testing has not been validated for cadaveric nasopharyngeal samples with varying rates of false negative results and may provide a false sense of security. With the above concerns, additional NP swabbing was resorted to in 59 cornea donors to rule out the presence of SARS-CoV-2 in the deceased, especially with the ongoing pandemic. All the donors had the a cause of death unrelated to respiratory symptoms of COVID. In total, 18.64% of these donors were positive on NP swabs for COVID-19 on RT-PCR. These donors might have been asymptomatic carriers, died due to undiagnosed COVID-19, or were persistently RT-PCR positive despite the resolution of undiagnosed COVID-19. Because none of the donors had a COVID-19–positive report before death, the status of COVID-19 before death cannot be commented on. Not all patients with COVID have been reported to have respiratory symptoms and may have varied and unpredictable symptoms or may be asymptomatic.[17]

These donors were detected negative for the SARS-CoV-2 antigen by rapid antigen testing before in situ excision. This may possibly signify low sensitivity of the test in cadaveric samples. Bullard et al[19] concluded in their study that the Ct value of ≥24 for an E gene RT-PCR may predict the lack of infectivity from respiratory samples in a clinical and community context. In a retrospective study conducted by Magleby et al,[20] Ct values from an RT-PCR chain reaction assay applied to nasopharyngeal swab samples were classified with a high-viral load (Ct < 25), a medium-viral load (Ct 25–30), and a low-viral load (Ct > 30). With 54.54% of COVID-positive donors having Ct values <25 and potentially highly infectious, and an additional 18.18% of donors with Ct values between 26 and 30 having a medium-viral load, there is definite risk of transmission of COVID-19 infection to the personnel retrieving the corneas. Risk of transmissibility of COVID-19 through corneal transplantation of corneas retrieved from these donors is not clear. Further microbiological studies are needed to detect SARS-CoV-2 in the corneas retrieved from donors found to be positive for COVID-19 on routine donor testing. With currently available

evidence and limited test kits, PCR testing is not considered mandatory for donor selection.

Until the availability of definite evidence showing lack of transmission of SARS-CoV-2 through corneal transplantation, nasopharyngeal swabbing is recommended for all the deceased during corneal retrieval. Corneas should be released for transplantation purposes only after the NP swabs test negative from the donor who died from causes unrelated to COVID. More studies are needed to assess SARS-CoV-2 PCR in cadaveric donor samples and donor storage medium.

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