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LETTER TO THE EDITOR

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Conjunctiva is not a preferred gateway of entry for SARS-CoV-2 to infect respiratory tract

To the Editor,

We read with interest the article of Xia et al[1] on the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in tears and conjunctival secretions from patients with novel coronavirus disease (COVID-19). Xia et al[1] reported that conjunctivitis was found only in one patient out of 30 cases with confirmed COVID-19, and SARS-CoV-2 RNA was detected in tears and conjunctival secretions by real-time polymerase chain reaction (RT-PCR) only in one patient complicated by conjunctivitis, whereas conjunctival swab samples from the other 29 patients with COVID-19 were all negative for SARS-CoV-2. In the discussion, Xia et al[1] implicated that SARS-CoV-2 might transmit through conjunctival tissue although it was not a common route of transmission. However, our opinion is that SARS-CoV-2 is unlikely to be transmitted via the conjunctiva route. The conjunctiva is neither a preferred tissue for SARS-CoV-2 infection nor is a preferred gateway of entry for SARS-CoV-2 to infect respiratory tract, based on previous clinical and experimental investigations including the findings of Xia et al.[1]

The conjunctival mucosa is directly exposed to infectious droplets expelled by patients during close contact and fomites when the eye is touched by contaminated hands.[2,3] Moreover, the mucosa of the conjunctiva and upper respiratory tract is connected by nasolacrimal duct and shares the same entry receptor of SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2), on host cell membranes.[2,3] Hence, it is reasonable to postulate that the conjunctiva may be easily involved in SARS-CoV-2 infection, and may even act as a route of transmission during SARS-CoV-2 infection. However, until now, viral conjunctivitis has been reported in literature only in five cases with COVID-19, whereas in zero and four cases infected by SARS-CoV and human coronavirus-NL63 which share the same entry receptor (ie, ACE2) with SARS- CoV-2, respectively (Table 1).[1-3] Moreover, SARS-CoV-2 RNA tested in tears and conjunctival secretions by RT-PCR is positive only in three cases and probable positive in two cases with COVID-19, whereas positive only in three cases with SARS-CoV infection. All samples with positive RT-PCR test results were collected within 9 days (mean 5 days) after the onset of the infection (Table 1).[1,2]

Taken together, recent clinical evidence and laboratory test results suggest that the conjunctiva is rarely involved in SARS-CoV-2 infection, and that the conjunctiva is neither a preferred tissue for SARS-CoV-2 infection nor is a preferred gateway of entry for SARS-CoV-2 to infect respiratory tract.[2] We agree with Peng and Zhou[4] that the premise for transmission through conjunctiva must be based on that SARS-CoV-2 can replicate, and cytopathic changes and viral particles could be identified in conjunctival epithelial cells.

The rarity of viral conjunctivitis in SARS-CoV-2 infection may exist in three interpretations: first, the expression of ACE2 protein on conjunctival epithelial cell membranes is much less than that in human lung and kidney tissues.[2,5,6] Second, the binding capability of ACE2 protein on conjunctival epithelial cells to SARS-CoV spike protein is much lower than that in lung tissues.[2,7] Third, the protective effect of the antimicrobial agents in tears including lactoferrin and secretory Immunoglobulin A (IgA), and constant tear rinsing on ocular surface which could eliminate the viruses dropped onto ocular surface into the nasal cavity through nasolacrimal duct.[2,8] Previous investigations have revealed that the binding of SARS-CoV to its entry receptor, ACE2 protein, depends on the assistance of an attachment receptor, heparan sulfate proteoglycans, on host cell membranes.[9] Lactoferrin can inhibit the binding of SARS-CoV to ACE2 protein by preventing the attachment of SARS-CoV to heparan sulfate proteoglycans.[2,8,9]

The extremely low positive rate of SARS-CoV-2 tested by RT-PCR in tears and conjunctival secretions from patients with COVID-19 may be interpreted as follows: first, current RT-PCR test for SARS-CoV-2 RNA is not sensitive enough, its sensitivity generally ranges from 50% to 60%.[2] Second, sample collecting time is in the later phase of viral infection. Previous investigations revealed that samples with positive PCR results were all collected within 9 days (mostly within 7 days) after the onset of the infection.[1,2] SARS-CoV-2 in tears and conjunctival secretions may come from the secretion of lacrimal glands and conjunctival epithelial cells, or even fomites transmitted onto ocular surface via splashed droplets or via direct touch by contaminated hands. Host immune system can be activated and leads to a significant increase in lactoferrin and secretory IgA levels in tears and in circulating Immunoglobulin M level in plasm on days 3 to 5, and in circulating Immunoglobulin G level in plasm on days 10 to 15 after virus inoculation or infection.[2,8] Since viremia contributing to virus secretion from lacrimal glands may only present for a short time during the early phase of viral infection,[4] and conjunctival epithelial cells is not susceptible to SARS-CoV-2 infection. SARS-CoV-2 RNA may present in tears and conjunctival secretions only in the early phase of the infection.

Most recently, Deng et al[10] reported that asymptomatic SARS-CoV-2 infection could be induced by conjunctival inoculation in a cynomolgus macaques model, and that SARS-CoV-2 was detected in conjunctival swabs only on the first day after conjunctival inoculation, whereas continuously detected in nose swabs and throat swabs from 1 to 7 days after conjunctival inoculation. This finding suggested that the virus load in the mucosa of nasal cavity and throat was much higher than that in conjunctiva. Hence, it is more likely that

TABLE 1 Ophthalmic manifestations and laboratory results of human coronavirus infection

No.	Author	Year	CoV species	Number of patients (confirmed/suspect)	Number of patients with viral conjunctivitis	CoV RNA tested in tears and conjunctival secretions	Conjunctival swabs sampling time after the onset of CoV infection
1	Loon	2004	SARS-CoV	36 (8/28)	0	3 cases positive	Days 3, 4, and 9, respectively
2	Chan	2004	SARS-CoV	20 (17/3)	0	All negative	6, 8, and 3 cases in the 1st, 2nd, and 3rd wks, respectively
3	Leong	2004	SARS-CoV	64 (64/0)	0	All negative	In convalescent phase
4	Yuen	2004	SARS-CoV	45 (45/0)	0	NA	NA
5	Hoek	2004	CoV -NL63	1 (1/0)	1	NA	NA
6	Vabret	2005	CoV -NL63	28 (28/0)	3	NA	NA
7	Dai	2020	SARS-CoV-2	1 (1/0)	1	NA	NA
8	Zhou	2020	SARS-CoV-2	67 (64/3)	1	1 case positive 2 cases probable positive (negative for the patient with conjunctivitis)	NA
9	Sun	2020	SARS-CoV-2	72 (72/0)	2	1 case positive	Day 6 for CoV positive case (sampled on days 10, 19, and 21 is all negative) NA for CoV negative case
10	Xia	2020	SARS-CoV-2	30 (30/0)	1	1 case positive	Days 3 and 5 (sampled twice for the same patient)

Abbreviations: CoV, coronavirus; NA, not available; RNA, ribonucleic acid; SARS-CoV, severe acute respiratory syndrome coronavirus.

SARS-CoV-2 infection is transmitted via the nose and throat route than via the conjunctiva route in this animal model. SARS-CoV-2 exposed to the ocular surface might first be transported to nasal and nasopharyngeal mucosa by constant tear rinsing through lacrimal duct, and then cause respiratory tract infection. Similar findings were reported in a cynomolgus macaques model using conjunctival and nasal inoculation with SARS-CoV virus.[2]

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