Review Article

Comparison of the transmission and detection potential of COVID-19 virus and other viruses in tear fluids

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ABSTRACT

The COVID-19 virus is a subgroup of zoonotic viruses. The most terrible problem started when the number of infected patients with acute respiratory syndrome quickly spread around the world, which made it as a global pandemic. This novel virus could transmitted from person to person via infected droplet entering the respiratory system whether nasal or oral cavity. Close contact less than 6 feet, with infected individuals particularly in crowded environments has characterized the rapid spread of the infection. Clinical signs of the COVID-19 infection have mentioned the presence of some ocular findings such as conjunctival congestion, conjunctivitis and even corneal injury associated with the classical COVID-19 infection. In this review, we showed that different viruses could be and transmitted by tear fluid which encourage us to search regarding to this potential in COVID-19 virus.

***Keywords*:** Transmission, detection, COVID-19, virus, tear

INTRODUCTION

Coronaviruses are enveloped, non-segment and positive-sense RNA which belong to the family of coronaviridae. Recently, another kind of coronavirus was the headlines of important news and reports which caused the specific pneumonia cases in Wuhan, Hubei, China, since December-2019 [1].

On January 31, 2020, the World Health Organization (WHO) explained the outbreak of coronavirus as a public health emergency of global worrying [2]. On February 11, 2020, the WHO officially named the infection due to the virus as coronavirus disease 2019 (COVID-19) [3].

A team of coronavirus study in global level on taxonomy of viruses named the etiologic agent of COVID-19 as "severe acute respiratory syndrome that referred to Coronavirus. On March 11, 2020, the WHO declared COVID-19 a pandemic [4]. On December 17, 2020, the total COVID-19 fatalities are 1,643,339 and the all confirmed infected cases are 72,854,747 in all over the world [5].

The respiratory problems that are caused by COVID-19 are completely described, though the ophthalmological implications of syndrome have not yet been explained clearly [3]. The authors of a recent study concluded that one-third of patients with COVID-19 had ocular abnormalities. Though, the prevalence of SARS-2 in patients with severe disease in tears is low and transmission of COVID-19 in ocular secretion is possible [6].

Regarding to issuing of more reports in literature associating coronavirus and ophthalmologic problems the aim of this research is comparison of transmission, detection patients of COVID-19 virus with other viruses in tear fluids and possible treatment of the ocular disease.

COVID-19 virus overview and biology

COVs is one of the great member of Coronaviridae family which have four types include: α, β, γ and δ coronaviruses. COVs is single stranded positive sense RNA virus with 30 Kb length of genome, which its RNA genome could code both of structural proteins (SPs) and nonstructural proteins (NSPs) [7]. All COVs have different parts which were made include: spike, membrane, envelope and neoclocupsid that could affect a wide range of birds and mammals [8,9]. WHO declaration introduces the coronavirus disease 2019 as public health emergency of international concern. Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) which cause COVID-19, coronavirus spread primarily through droplet and saliva or discharge from the nose and when infected person talks, coughs or sneezes [10]. Transmission from person to person in close contact or touching a surface or an object that has viruses containing respiratory droplets and then touching the eye, mouth and nose [11]. Coronaviridae family is responsible for the outbreak of two epidemics in last 20 years, one zoonotic and second respiratory syndrome. In 2002-2003 COVs were responsible for the severe acute respiratory syndrome (SARS) [12-14]. and in 2012 of the middle east respiratory syndromes (MERS) [15]. Altogether they caused more than 10,000 cumulative cases with mortality rates of 10 % for SARS-Cov, and 37 % for MERS-Cov and because of coronavirus pandemic on February 11, 2020 the WHO officially named the infection due the viruses as coronavirus disease 2019.

Clinical symptoms of COVID-19 infection

SARS-CoV-2 infected person could be asymptomatic or present with mild symptoms. Approximately 80 % of patients should have mild to moderate disease and suppose to cure without major problems which are reported in WHO-China joint mission report on 28th February 2020. The incubation period for that severs ones is 1 to 15 days, and infected person could develop symptoms up to 28 days after infection [16]. Typical symptoms of COVID-19 infection are fever >38 ºC, sore throat, non-productive cough, myalgia, shortness of breath, skin rashes, anosmia, ageusia, headaches and fatigue also lymphopenia, thrombocytopenia and abnormal liver studies [17-19]. A small percentage of infected individuals with SARS-CoV-2 presents with conjunctivitis, which could indicate ocular manifestations with this novel virus [20]. A very high number of pathophysiological problems of the infection are associated with a significant immune response provoked by the virus [21].

Different kind of virus's detection methods

According to the Table 1, the methods of sampling for ocular secretions are include direct conjunctival swabs, schirmer's test strips and glass capillary micropipettes, which are used in a previously cases to detection of SARS-Cov-2 by RT-PCR successfully [22] but the results of a more recent study of 17 patients shown no evidence of SARS-Cov-2 shedding in tears samples by same sampling methods [23]. In an another case [24] a 65 years old woman who had returned to Italy from Wuhan in China was admitted to hospital with some symptoms like non-productive cough and sore throat and more among symptoms conjunctivitis persisted until 16 day after onset of symptoms and ocular swab were positive for viral RNA until 21 days after hospital admission but Interestingly test of ocular swab was undetectable just one day after it [25]. One study collected 64 tear samples by using schirmer strip test from 17 confirmed Covid-19 patients and analyzed the tears using PCR to detect viral RNA [23] all during three weeks of infection which was no viral RNA detection in all nasopharyngeal swabs. All of these define that transmission by tear or conjunctival secretions may appear only in 5 % of all cases of SARS-Cov-2.

Table 1. Different kind of viruse transmission potential by tear fluid

Virus	Specie	Sample	Collection method	Disease	Study method	Result	Year	Ref
COVID-19	Human	Tear fluid	-	Patients with COVID-19	RT-PCR	COVID-19 nucleic acids were not detected in tear samples from 10 positive patients.	2020	[26]
SARS-CoV-2	Human	Tear fluid	Swabbing.	COVID-19 patients with obstruction of common lacrimal duct	RT-PCR, Next-Generation Sequencing (NGS)	SARS-CoV-2 Ag was positive in one eye for 2 weeks more after nasopharyngeal swab became negative.	2020	[27]
SARS-CoV-2	Human	Tear fluid	Conjunctival swabbing	Patients with COVID-19	RT-PCR	All tear samples showed negative results, even when nasopharyngeal swab samples continued to show positive results.	2020	[23]
SARS-CoV-2	Human	Tear fluid	Conjunctival swabbing	Patients with COVID-19	RT-PCR	2 of 12 patients (16.7 %) with ocular abnormalities had positive results for SARS-CoV-2 on RT-PCR From conjunctival swab.	2020	[6]
SARS-CoV-2	Human	Tear fluid	Conjunctival swabbing	Patients with COVID-19	RT-PCR	Conjunctival swabs did not identify SARS-CoV-2 by RT-PCR.	2020	[28]
SARS-CoV-2	Human	Tear fluid	Swabbing.	Patients with COVID-19	RT-PCR	Three (7 %) of tear samples were positive for SARS-CoV-2.	2020	[29]
SARS-CoV-2	Human	Tear fluid	Conjunctival swabbing	Patients with confirmed novel coronavirus pneumonia (NCP)	RT-PCR	2 of 30 samples of tear and conjunctival secretions were obtained from the only one patient with conjunctivitis yielded positive RT-PCR results. Fifty eight samples from other patents were all negative.	2020	[22]
SARS-CoV-2	Human	Tear fluid	Conjunctival swabbing	Patients with confirmed SARS-CoV-2 infection	RT-PCR	From 40 patients, one was found positive by conjunctival swab RT-PCR, and nine were found negative.	2020	[30]

SARS-CoV	Human	Tear fluid	Conjunctival swabbing	Patients who were suspected of having SARS	RT-PCR	3 of 36 patients with probable SARS (one female and two male patients) had positive results from their tear samples.	2004	[31]
SARS-CoV	Human	Tear fluid, conjunctival cells	Tear swab and conjunctival scraping	Patients with confirmed SARS	RT-PCR	In all tear and conjunctival scraping samples, no SARS-CoV virus could be detected by RT-PCR.	2004	[32]
2019 novel corona virus (2019-nCoV)	Human	Conjunctival swabs	Swabbing.	Patients with NCP	RT-PCR	Conjunctival swab samples from 1 of 63 NCP patients yielded positive PCR results and 2 NCP patients yielded probable positive PCR results. Conjunctival swab samples from the four suspected cases of NCIP were negative.	2020	[33]
Hepatitis C (HCV)	Human	Tear fluid	Collected with micro capillary tubes.	HCV positive patients	RT-PCR	All 76 patients chronically infected with HCV were positive by RT-PCR for tear fluid and plasma.	1995	[34]
HCV	Human	Tear fluid, Aqueous Humor	Collected by a micropipette.	Patients with Anti-HCV Antibody Positive Who Underwent Cataract Surgery	RT-PCR, ELISA	Viral load detected in aqueous humor and tear fluid samples was considerably lower compared to the serum samples.	2014	[35]
HCV	Human	Tear fluid	Collected with micro capillary tubes.	Patients With Chronic Hepatitis C	RT-PCR	HCV RNA was detected in 9.8 % (5/51) of the tear fluid samples.	1997	[36]
HCV	Human	Lacrimal fluid	-	Patient with recurrent corneal peripheral ulcer	RT-PCR	HCV RNA was detected in lacrimal fluid of patient with recurrent corneal peripheral ulcer.	2001	[37]
Transfusion-transmitted	Human	Tear fluid, Aqueous Humor	Collected from conjunctiva sac with a hematocrit	Patients undergoing planned cataract surgery	RT-PCR	TTV DNA was detected in serum, tear and aqueous humor of patients undergoing cataract surgery	2007	[38]

virus (TTV)			tube after conjunctival stimulation with a cotton tip.					
Herpes simplex virus (HSV)	Human	Tear fluid	Collected from bilateral conjunctival sacs by pipette.	Herpetic stromal keratitis and persistent epithelial defect patients	RT-PCR	A relatively high level of HSV-DNA, was detected in the tear samples of these two disease forms, although the source of the viral replication was not identified.	2008	[39]
HSV	Human	Tear fluid	Collected from the lower fornixes of both eyes into capillary tubes.	Stromal Herpes Simplex Keratitis (HSK)	ELISA	The tear sIgA–positive rate was 36.59 % in stromal Keratitis, whereas none of the controls were found as sIgA positive.	2013	[40]
HSV-1	Human	Tear fluid	Collected from the lower fornix using schirmer strips for 5 minutes.	Patients with HSK	RT-PCR	HSV DNA was detected in 23 out of 115 (20 %) tear samples.	2013	[41]
HSV-1	Human	Tear fluid, corneal scrapings	Collected from the lower conjunctival fornix with Schirmer strips and borosilicate glass capillaries	Patients with Viral keratitis	RT-PCR, indirect immunofluorescence assay	Corneal scrapings yielded a significantly better HSV positivity than tears in both the PCR assay and immunofluorescence assay.	2014	[42]
HSV-1	Human	Tear fluid	Collected by sno-strips placed on conjunctiva	HSV-1 seropositive patients	RT-PCR, western blot	HSV-1 was rarely found in tears.	2016	[43]

HSV	-	Tear fluid	Synthetic tear	Patients with Conjunctivitis	Surface enhanced Raman scattering (SERS)	SERS could potentially be used to detect the presence of HSV particles in an aqueous solution such as the tear film.	2008	[44]
HSV-1	Human	Tear fluid	Collected by filter paper	Patients with Bell's Palsy	RT-PCR	HSV-1 deoxyribonucleic acid was detected in 38 specimens (11.8 %) from 5 patients (31 %).	2002	[45]
Measles Virus	Human	Tear fluid	Collected by a Schirmer's strip	Patients with Measles keratitis	RT-PCR	We demonstrated the presence of measles virus genomic RNA in the tears of a patient with measles keratitis.	2002	[46]
Varicella zoster virus (VZV)	Human	Tear fluid, Saliva	Collected by attaching a paper filter to each of the lower eyelids and al-lowing the filters to absorb fluid.	Patients with Ramsay Hunt Syndrome	RT-PCR, Microplate hybridization	Secretion of varicella zoster virus DNA into the tear fluid and saliva was confirmed	2000	[47]
HSV 6 and VZV	Human	Tear fluid	Collected with micro-capillary tube from the lower fornix.	Patients with Bell's Palsy	RT-PCR	HHV-6 DNA can be detected in the tear fluid of a significant number of Bell's palsy patients. Also, we found VZV DNA in tear fluid samples from two Bell's palsy patients, showing that VZV can be detected in the tear fluid of patients with Bell's palsy without cutaneous vesicles.	2000	[48]
Cytomegalovirus (CMV)	Human	Tear fluid	Collected with glass capillary micropipettes from the inferior temporal tear meniscus	Patients with CMV infection and retinitis	ELISA	There was a strong association between high tear levels of anti-CMV antibodies and active ocular infection.	2006	[49]

Epstein-Barr virus (EBV)	Human	Tear fluid	Collected by a Schirmer's strip	Sjogren's Syndrome and HIV	RT-PCR	EBV-1DNAwas found in the tear film of 4 patients with Sjogren's syndrome and 12 patients with HIV infection had evidence of EBV-1 in their tears.	2002	[50]
Bronchitis virus Massachusetts strain M41	Chicken	Tear fluid	Collected by micropipettes or on filter paper.	Four-week-old specific-pathogen-free chickens	ELISA	Virus-specific immunoglobulin (Ig) A and IgG were detected in the tear fluid.	2005	[51]
Zika virus (ZIKAV)	Human	Conjunctival fluid	Swabbing.	ZIKAV-infected patients	RT-PCR	Samples from 3 of 29 patients were found positive by qRT-PCR for ZIKAV.	2017	[52]
ZIKAV	Mouse	Tear fluid	Collected after gentle lavage with 10 µl of PBS using FP plus multiflex tips.	Ifnar1$^{-/-}$ mice	RT-PCR	Detected abundant viral RNA in tears, could suggesting that virus may be secreted from lacrimal glands or shed from the cornea.	2016	[53]
HIV-1 virus	Human	Tear fluid	Collected into sterile tubes from patients' eye directly	HIV-1-infected patients	RT-PCR	HIV-1 viral load in tears was detected positively in all of patients.	2011	[54]
HIV-1 virus	Human	Tear fluid	Collected into sterile tubes from patients' eye directly	AIDS patients	bDNA Analyzer System 340	HIV viral load in tears was detected.	2019	[55]
HIV-1 virus	Human	Tear fluid	Collected using Schirmer's strips	Dry eye patients with HIV infection	RT-PCR	HIV viral load in tears was detected in some patients.	2017	[56]
Adeno virus	Human	Tear fluid, conjunctival cells	Tear film washes, filter paper, and swabbing	Patients with a history of adenovirus conjunctivitis	RT-PCR	Evidence of adenovirus DNA was detected in 17 of 30 patients.	2005	[57]

Continuous studied and researches regarding to the feasibility of PCR to detect the viruses has been performed, since the sensitivity of PCR assays for the detection of the DNA of infectious microorganisms has been shown to be very high, which indicates that this method is an effective and valuable laboratory tool [58]. In 1990, the first report came out that HSV DNA was detected in the cornea by PCR assay [59]. Thereafter, reports with small numbers of the cases were documented and these suggested that PCR is highly sensitive to herpes keratitis in tear and corneal scraping samples [60].

According to relationship between nasal cavity and eye by nasolacrimal duct, maybe there is a way to transfer viruses which are present in nasal cavity to eye. This reason should be a good idea to study potential of detection of viruses especially COVID-19 in tear fluids. According to table 1 which showed the results of different viruses' detection in tear fluids by PCR method, we can consider a chance for detection COVID-19 virus in tear fluids. On the other hand, the sampling method by tear fluid should be easier than nasopharynx swabbing for patients and medical staff.

It is known that the SARS-COV-2 can transfer with direct and indirect contact with mucous membrane in the eyes, nose and mouth [11,22]. Detection of COVID-19 in the ocular area SARS-CoV-2, SARS-CoV and some other corona viruses may be able to show in the ocular surface as the result of:

A. The conjunctiva being the direct insemination site of the virus from the infected droplets;
B. Migration through the nasolacrimal duct when there is the upper respiratory tract infection; or
C. Secretion via conjunctiva vessels during the disease.

Methods for sampling of ocular fluids are include; direct conjunctival swabs, schirmer's test strips, and glass capillary micropipettes. The main detective protocol for the viruses is the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), viral culture and Cytopathic Effects (CPE). The results of a study showed that the SARS-CoV-2 was detected by RT-PCR from a patient tears fluid [22]. A very most recent study from 17 patients has found no proof of SARS-CoV-2 that is shedding in the tears fluid [23]. There is probably a low risk of SARS-CoV-2 transmission through tears, but different results are due to differences in the volume of tears collected, techniques of sample collection, or timing of sample collection. In a prospective study, Xia et al. evaluated the conjunctival secretions of 30 confirmed cases of COVID-19 [22] and showed that just one of patients had conjunctivitis with SARS-CoV-2 positive. Likewise, Liang et al. [61] and Wu et al. [6] achieved positive results while in study by Liang et al. the patient with nucleic acid test positive did not have conjunctivitis [61]. A lot of the other studies also examined COVID-19 patients in which they are not reported to have ocular signs and symptoms. Another study examined 64 tear fluid samples in which they used schirmer's strip from 17 confirmed COVID-19 patients and studied the tears using PCR to detect viral RNA [23]. The samples were gathered in three weeks from the infection in the patients. When the viral RNA would be detected in all nasopharyngeal swabs, none of the virus was grown from the tears fluid samples and also no viral RNA would be detected, even from those patients which have the symptoms of the upper respiratory tract infection [33]. An another study tested 63 confirmed COVID-19 patients and four suspected patients with the PCR for viral RNA with conjunctival samples was tested positive with PCR, and the only patient with conjunctivitis was negative [33].

CONCLUSION

The pandemic situation which caused by the coronavirus SARS-CoV-2 has challenged biological scientific to find the means to control it. Large amounts of information about the agent have been collected related to the clinical infection. The immune response that ensues but we are still searching for better diagnostic tests therapeutics to control it and ultimately vaccines which finally was found by different companies.

Several models of viral transmission regarding the ocular structures as possible transmission routes have been described. The findings described correlated with clinical findings have only begun to open a small understanding of the ocular manifestations of COVID-19 infection. If the virus can enter through the conjunctiva it will be necessary to advise precautions in contact lenses wearing and extend the precaution about eye touching. It is necessary to set up protocols for the evaluation and testing of the ocular samples as tear and conjunctiva of suspected infections information provided by the scientific associations of visual health care professional will continue to strengthen the preventive measures to

minimize the risk contagion in professional and patients.

Analyzes of other viruses transmission potential through tear fluid showed that there is a great way to detect and transfer of viruses by tear fluids, but regarding to COVID-19, there was no confirm and strong results that showed and make sure the potential of tear fluids to detect COVID-19 virus.

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