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AKADÉMIAI KIADÓ

Presence of SARS-CoV-2 on the conjunctival mucosa in patients hospitalized due to COVID-19: Pathophysiological considerations and therapeutic implications

ANDRÁS VÉGH[1*], ANITA CSORBA[1], ÁKOS KOLLER[2,3], BEHNAM MOHAMMADPOUR[1], PETRA KILLIK[1], LILLA ISTVÁN[1], MÁRTON MAGYAR[1], TERÉZ FENESI[1] and ZOLTÁN ZSOLT NAGY[1]

[1] Semmelweis University, Department of Ophthalmology, Budapest, Hungary

[2] Semmelweis University, Translational Medicine Institute, Budapest, Hungary

[3] Hungarian University of Sports Sciences, Research Center for Sports Physiology, Budapest, Hungary

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in a worldwide pandemic, due to its great capacity to invade the human body. Previous studies have shown that the primary route of invasion of this virus is the human respiratory tract via the co-expression of ACE2 receptor and TMPRSS2, a serine protease on the cellular surface. Interestingly, this condition is present not only on the respiratory epithelium but on the conjunctival mucosa, as well. Thus, we hypothesized that SARS-CoV-2 is present on the conjunctival mucosa. *Aim:* To prove that SARS-CoV-2 can be detected in the conjunctiva. *Methods:* Previously nasopharyngeal swab-sample based real-time polymerase chain reaction (PCR) positive COVID-19 infected patients were selected at the COVID Care Centers of Semmelweis University, Budapest, Hungary. The study was approved by the ethical committee of Semmelweis University. During their recovery, both nasopharyngeal and conjunctival swab-samples were taken and PCR method was used to detect the presence of SARS-CoV-2 RNA. Appropriate statistical analysis was performed. *Results:* The study population consisted of 97 patients, 49 females (50.5%) and 48 males (49.5%), with a mean age of 67.2 ± 11.9 years. During recovery, with nasopharyngeal swabs, the PCR test was positive in 55 cases (56.70%), whereas

* Corresponding author. Mária utca 39, Budapest H-1085, Hungary. E-mail: vegh.andras@med.semmelweis-univ.hu

with conjunctival swabs it was positive in 8 cases (8.25%). Both tests were positive in 5 cases (5.15%). In some patients, ocular symptoms were observed as well. The rest of the patients (29 cases) had negative nasopharyngeal PCR tests during recovery. *Conclusions:* Although only in few cases, the data of the present study provides a proof of concept that SARS-CoV-2 can be present on the conjunctival mucosa even in nasopharyngeal negative patients, a finding, which can have clinical importance. Also, on the basis of these findings one can hypothesize that - in addition to the respiratory tract – the conjunctiva can be an entrance route for SARS-CoV-2 to the human body. Thus, in high-risk conditions, in addition to covering the mouth and nose with mask, the protection of the eyes is also strongly recommended.

KEYWORDS

COVID-19, SARS-CoV-2, conjunctiva, ocular symptoms, polymerase chain reaction, PCR

INTRODUCTION

The COVID-19 pandemic

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been the greatest worldwide pandemic since the Spanish flu in 1918–1919 [1]. It has greatly impacted the national and global healthcare systems and demanded over 4 million lives out of more than 190 million people infected [2]. The primary entrance location of SARS-CoV-2 infection is in the mucosal cells after being exposed to the virus directly or indirectly and facilitated by the binding of coronavirus spike protein subunit to the angiotensin-converting enzyme 2 (ACE2) receptor [3–15]. The cellular entry also requires the priming of the protein by a serine protease, TMPRSS2, or other proteases [11, 15]. Thus, the co-expression of ACE2 and TMPRSS2 on the cellular surface are necessary for the cellular infection, a condition, which is present both on the epithelium of the respiratory tract and the conjunctival mucosa [11, 15].

Pulmonary and extra-pulmonary manifestations

COVID-19 is best known for its respiratory and systematic symptoms that manifest from asymptomatic infection to acute respiratory distress syndrome (ARDS) [7, 15]. Classical symptoms are fever, cough, fatigue, weakness, loss of olfaction and taste [15, 16]. In addition to the respiratory symptoms, this disease can affect other tissues and organs, such as hematologic-, cardiovascular-, gastrointestinal-, hepatobiliary-, endocrine-, dermatological-, neurological- and ophthalmological systems [15]. This latter one is not always appreciated and sometimes overlooked [17].

Previous ocular findings

As mentioned above, the conjunctival mucosa also can be exposed for SARS-CoV-2 infection, which than can elicit various ocular symptoms. The most common ocular manifestation of COVID-19 is conjunctivitis with epiphora, conjunctival congestion or chemosis, itching, foreign body sensation and/or watery discharge [7–28]. Other pathologies and subjective symptoms as blurred vision, tarsal petechiae, hemorrhage or pseudomembrane, mucous filaments, anterior or posterior uveitis, retinal microangiopathy, microhemorrhages, cotton-wool dots and neuro-

ophthalmologic manifestations such as diplopia, ophthalmoparesis and Miller-Fischer syndrome can also frequently occur [7–28]. All of these underlines the importance of extending medical attention and care to the ocular system as well, during COVID-19 infection.

Hypothesis and aim

Because of the aforementioned, we hypothesized that SARS-CoV-2 is present on the conjunctival mucosa and accompanied with ocular syndromes. Thus, we aimed to prove that SARS-CoV-2 can be detected in the conjunctiva in patients hospitalized due to COVID-19 and to identify ocular symptoms. [3, 4, 11–16, 18–21].

PATIENTS AND METHODS

The patients included in the present study provided an informed consent for the investigation, which was approved by the ethical committee of Semmelweis University. Patients had been previously admitted with SARS-CoV-2 positive nasopharyngeal swab to the COVID Care Centers of Semmelweis University, Budapest. During recovery, the conjunctival swab test was performed within 24 h after the control nasopharyngeal swab. There were 97 patients examined between January and April of 2021.

During recovery, both nasopharyngeal and conjunctival swabs were taken and analyzed with real-time polymerase chain reaction (PCR) in order to show the presence of SARS-CoV-2 RNA in the sample (in the Central Laboratory of Department of Laboratory Medicine, Semmelweis University). Conjunctival samples were taken from both eye (inferior conjunctival fornix) with synthetic fiber swabs and placed into the same test tube, meaning that infection on only one eye caused PCR positivity. Vital (body temperature, blood pressure, pulse, oxygen saturation, the amount of oxygen supplementation, respiratory rate) and laboratory parameters (CRP, PCT, IL-6, ferritin, blood count, renal and liver function, necro-enzymes and D-dimer) as well as symptoms (e.g., presence of fever or cough) were routinely recorded during the admission of the patient.

In some patients in the COVID-19 Centers, ophthalmological history was taken and basic examination was performed.

STATISTICS

Statistical analysis was performed using IBM® SPSS® Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). All continuous variables were expressed as mean, median and interquartile ratio (IQR). Kolmogorov-Smirnov test was used to assess normality. Mann-Whitney test was used for comparison of continuous variables. Categorical variables were summarized as counts (percentages). Fisher's exact test was used for comparison of categorical variables. In all analysis, the level of significance was considered at $P < 0.05$.

RESULTS

The mean age of the patients was 67.22 ± 11.91 ($n = 97$) years; 49 (50.52%) of them were females, 48 (49.48%) were males. The anamnestic, vital and laboratory parameters as well as symptoms of patients occurring are included in Table 1.

Table 1. Anamnestic, vital and laboratory parameters presented in the group of patients, the level of $P < 0.05$ considered as statistically significant

	Conjunctival swab			Ocular symptoms		
Parameter	PCR− ($n = 89$)	PCR+ ($n = 8$)	P	Absent ($n = 91$)	Present ($n = 6$)	P
Age (years)*	67.64 (median 69, IQR 59–78)	62.50 (median 58.5, IQR 52.5–67.25)	0.276	67.03 (median 68, IQR 55.5–78)	70.00 (median 71, IQR 63.75–76)	0.824
Hypertension in anamnesis**	73.03%	50.00%	0.223	83.33%	70.33%	0.669
Diabetes in anamnesis**	49.43%	87.50%	0.294	66.67%	47.25%	0.689
Other disease(s) in anamnesis**	4.49%	25.00%	1.000	83.33%	81.32%	1.000
Ophthalmologic anamnesis**	15.73%	12.50%	1.000	50.00%	13.87%	0.046
Fever**	29.07%	50.00%	0.246	16.67%	31.82%	0.662
Cough**	37.93%	25.00%	0.706	33.33%	37.08%	1.000
Other symptom(s)**	81.82%	87.50%	0.076	50.00%	84.44%	0.066
Days since the onset of symptom(s)*	14.80 (median 12, IQR 8–19)	13.50 (median 12, IQR 9.25–16)	0.818	14.47 (median 12, IQR 8–18)	18.00 (median 16, IQR 10–26.5)	0.505
Days since hospitalization*	8.63 (median 7, IQR 4–11)	7.00 (median 6.5, IQR 3.25–8.25)	0.562	8.21 (median 7, IQR 4–11)	11.33 (median 11.5, IQR 4.25–18.75)	0.537
Body temperature (°C)*	36.76 (median 36.79, IQR 36.5–37)	36.61 (median 36.6, IQR 36.3–36.95)	0.661	36.76 (median 36.7, IQR 36.5–37)	36.56 (median 36.6, IQR 36.6–36.6)	0.211
Systolic blood pressure (Hgmm)*	128.10 (median 125, IQR 112–144.5)	119.16 (median 118.5, IQR 112.25–126.75)	0.247	127.81 (median 125, IQR 110.75–143)	120.33 (median 117, IQR 113.25–123)	0.318
Dyastolic blood pressure (Hgmm)*	77.40 (median 77, IQR 70–84)	75.88 (median 73.5, IQR 68.5–84.5)	0.730	70.55 (median 77.5, IQR 69.75–84.25)	73.17 (median 70.5, IQR 68.25–74.25)	0.275
Pulse (1/min)*	84.00 (median 82, IQR 71–92.75)	91.75 (median 89, IQR 86–104.25)	0.121	84.77 (median 83.5, IQR 71–94.25)	83.00 (median 86, IQR 80–86.75)	0.932
Oxygen saturation (%)*	95.06 (median 96, IQR 94–97)	96.67 (median 69.5, IQR 96–97)	0.237	95.15 (median 96, IQR 94–97)	95.6 (median 96, IQR 95–98)	0.696
Oxygen supplementation (l min^{-1})*	3.24 (median 0, IQR 0–6)	1.83 (median 1, IQR 0–2.75)	0.696	3.23 (median 0, IQR 0–6)	1.6 (median 0, IQR 63, 0–2)	0.494
Respiratory rate (1/min)*	19.46 (median 18, IQR 17–20)	18.43 (median 20, IQR 16.5–20)	0.722	19.35 (median 18, IQR 17–20)	19.60 (median 18, IQR 18–20)	0.930
C-reactive protein (mg l^{-1})*	86.75 (median 77.7, IQR 29.6–121)	108.00 (median 95.1, IQR 32.7–173.425)	0.525	89.51 (median 83.7, IQR 29.3–129.75)	73.32 (median 60.75, IQR 38.725–101.75)	0.765
Procalcintonine (ug/l)	0.78 (median 0.09, IQR 0.05–0.245)	0.42 (median 0.05, IQR 0.025–0.48)	0.757	0.78 (median 0.08, IQR 0.05–0.26)	0.17 (median 0.1, IQR 0.04–0.16)	0.948
Interleukin-6 (pg ml^{-1})*	78.23 (median 41.62, IQR 14.825–78.025)	12.65 (median 12.625, IQR 10.3925–14.8975)	0.123	79.62 (median 41.62, IQR 14.545–80.53)	24.01 (median 18, IQR 13.66–28.35)	0.255

(continued)

Table 1. Continued

Parameter	Conjunctival swab			Ocular symptoms		
	PCR− (n = 89)	PCR+ (n = 8)	P	Absent (n = 91)	Present (n = 6)	P
Ferritine (ug/l)*	804.54 (median 612.5, IQR 297–993)	962.67 (median 613.5, IQR 243–1154.25)	0.970	843.35 (median 632, IQR 315–1.104)	427.60 (median 284, IQR 218–534)	0.150
Red blood cell count (T l^{-1})*	4.29 (median 4.27, IQR 3.86–4.81)	4.305 ±(median 4.2, IQR 4.125–4.6425)	0.990	4.30 (median 4.23, IQR 3.91–4.82)	4.20 (median 4.335, IQR 4.08–4.523)	0.846
White blood cell count (G l^{-1})*	7.68 (median 6.55, IQR 4.92–9.32)	6.78 (median 6.32, IQR 5.0375–7.5375)	0.591	7.85 (median 6.49, IQR 4.905–9.27)	8.04 (median 7.885, IQR 6.15–10.775)	0.559
Platelet count (G l^{-1})*	246.52 (median 224, IQR 180–288)	246.75 (median 231.5, IQR 205–259.75)	0.637	246.66 (median 216, IQR 178.5–299.5)	244.67 (median 236.5, IQR 232–249.25)	0.393
GFR (mlmin^{-1}1.73 m^2)*	66.09 (median 80.5, IQR 45.45–90)	66.63 (median 82.3, IQR 58.525–90)	0.973	66.52 (median 81.2, IQR 46.2–90)	60.30 (median 66.5 IQR 44.9–84.425)	0.397
Carbamide (urea) (mmol l^{-1})*	9.58 (median 6.7, IQR 4.9–10.1)	12.83 (median 5.4, IQR 4–11.25)	0.376	9.59 (median 6.5, IQR 4.85–10.35)	13.72 (median 7.15, IQR 5.75–8.625)	0.816
Creatinine (umol/l)*	128.87 (median 79, IQR 66–114)	142.75 (median 69, IQR 55.25–135.25)	0.491	128.59 (median 78, IQR 66–111.5)	151.50 (median 91, IQR 63.25–121)	0.863
Aspartate aminotransferase (U/l)*	50.30 (median 41, IQR 26–60)	48.25 (median 46.5, IQR 32.75–56.5)	0.673	50.51 (median 41, IQR 27–60)	44.50 (median 40.5, IQR 29.5–46.25)	0.771
Alanine aminotransferase (U/l)*	38.76 (median 30, IQR 15.5–45.5)	39.88 (median 36, IQR 19.75–50.25)	0.648	39.08 (median 30, IQR 16–47)	35.50 (median 27, IQR 13.75–41)	0.624
Gamma-glutamil transferase (U/l)*	81.65 (median 54, IQR 28–91)	88.50 (median 82, IQR 65.5–99.75)	0.273	84.32 (median 54, IQR 30.5–93)	52.00 (median 75, IQR 21.75–80.25)	0.403
Lactate dehydrogenase (U/l)*	340.18 (median 304, IQR 210.5–387.5)	341.67 (median 329.5, IQR 225–425)	0.781	340.34 (median 306, IQR 210.5–387.5)	339.50 (median 321, IQR 229.75–400.25)	0.756
Creatine kinase (U/l)*	315.85 (median 125, IQR 48.5–266)	167.83 (median 124, IQR 71.5–169)	0.746	314.50 (median 103, IQR 49.75–275.25)	114.75 (median 72.5, IQR 68–119.25)	0.619
D-dimer (ug/ml)*	1.70 (median 1.4, IQR 0.6975–2.455)	0.85 (median 0.65, IQR 0.52–0.83)	0.121	1.62 (median 1.6, IQR 0.89–2.645)	1.94 (median 1.6, IQR 0.89–2.645)	0.818

* Fischer exact test was performed with categorical parameters.

** Mann-Whitney test was performed with continuous parameters.

The nasopharyngeal swabs resulted in positive PCR test in 55 cases (56.70%), the conjunctival swabs were positive in 8 cases (8.25%), whereas both tests were positive in 5 cases (5.15%). The percentage distribution of the tests is depicted in Fig. 1.

In several patients in the COVID-19 Centers, ophthalmologic history was taken and basic examination was performed, the findings are included in Table 2. Six (6.19%) patients presented ocular symptoms, such as foreign body sensation (1 patient), acute glaucoma (1 patient), strabismus (1 patient), exophthalmos (1 patient), ectropium (1 patient) and ocular discharge (2 patients). Two of these patients were positive for conjunctival SARS-CoV-2 PCR test, and both presented ocular discharge; that is, 25% of the patients with positive conjunctival swab had ocular symptoms. Every ocular finding that had not been presented in the anamnesis was considered a symptom. Fifteen patients (15.46%) had ophthalmologic anamnesis, such as glaucoma (1 patient), pseudophakia (6 patients), retinal thrombosis (1 patient), central serous chorioretinopathy (1 patient), diabetic retinopathy (1 patient), endocrine orbitopathy (1 patient), cataract (1 patient), ptosis (1 patient) and age-related macular degeneration (AMD, 1 patient). Of the patients with

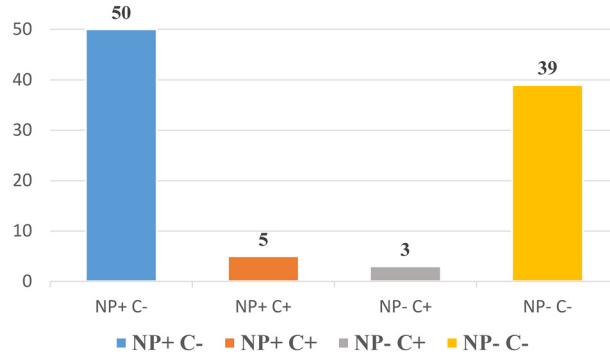

Fig. 1. Prevalence of positive and negative nasopharyngeal and conjunctival SARS-CoV-2 PCR results among the 97 patients investigated during recovery
NP: nasopharyngeal test, **C:** conjunctival test, whereas + and – signs indicate positive or negative SARS-CoV-2 PCR test, respectively.

Table 2. Summary of ocular symptoms and ophthalmologic anamnesis among the 97 patients investigated in COVID Care Centers at Semmelweis University

Ocular symptoms (6 cases)	Ophthalmologic anamnesis (15 cases)
1. foreign body sensation (1 case)	1. glaucoma (1 case)
2. acute glaucoma (1 case)	2. pseudophakia (6 cases)
3. strabismus (1 case)	3. retinal thrombosis (1 case)
4. exophthalmos (1 case)	4. central serous chorioretinopathy (1 case)
5. ectropium (1 case)	5. diabetic retinopathy (1 case)
6. ocular discharge (2 cases)	6. endocrine orbitopathy (1 case)
	7. cataract (1 case)
	8. ptosis (1 case)
	9. AMD (1 case)

AMD: age-related macular degeneration.

PCR positive conjunctival swab 12.50% had ocular symptoms and 50.00% of them had ophthalmologic anamnesis (Table 2).

Patients were divided in two groups in two ways: 1) whether they had ocular symptoms revealed during the examination and 2) if their conjunctival swab tested positive for SARS-CoV-2 PCR test. We have found that the only statistically significant difference between the two groups of patients with and without ocular symptoms was the prevalence of ophthalmologic anamnesis, which was significantly higher in patients with ocular symptom(s) (Table 1).

DISCUSSION

The salient finding of the present study is that – although only in few cases – conjunctival tissue sample was positive for COVID-19 in the absence of nasopharyngeal positivity.

During the study of 97 hospitalized elderly patients (~60 years old) with serious pulmonary and general COVID-19, symptoms were evaluated. Their majority required oxygen supplementation but none of them needed mechanical ventilation. In the present study 41 of all 97 patients required oxygen supplementation (42.3%).

At admission, the routine blood laboratory tests did not show significant difference between patients with or without ocular symptoms (Table 1). Also, there was no statistically significant difference between the vital and laboratory variables in patients with or without positive conjunctival SARS-CoV-2 PCR test result. Samples for PCR tests were taken from the conjunctival epithelium within 24 h after performing the control nasopharyngeal swab tests. Nasopharyngeal tissue sample showed positive SARS-CoV-2 PCR result in 55 cases (56.70%), whereas conjunctival swabs were positive in 8 cases (8.25%). The relatively low number of PCR positive nasopharyngeal swabs test is likely due to the fact that many patients were recovering from COVID-19 after 1–3 weeks of admission. Also, it is interesting to note that human conjunctival epithelium contains much less ACE2 receptors compared to the lower respiratory tract [11, 15]. Perhaps because of this reason, conjunctival swab can be PCR negative in spite of being a potential entrance route for SARS-CoV-2 virus.

Although conjunctivitis can be a present in COVID-19, previous findings of Bertoli et al. indicated that the exact incidence of conjunctivitis in COVID-19 patients is unclear, ranging between 0.8% and 31.6% [7]. In 2020, Güemes-Villahoz et al. reported that about 12% of COVID-19 patients presented conjunctivitis symptoms, which were associated with the disease and usually occurred in the early phase of the disease [19]. Moreover, Wu et al. have shown that in 73.7% of patients hospitalized due to COVID-19 had positive RT-PCR test for COVID-19 from the nasopharyngeal swabs, and of these, 5.2% of them yielded positive tests for SARS-CoV-2 in their conjunctival as well as nasopharyngeal specimens. A total of 31.6% of their patients had ocular manifestations consistent with conjunctivitis, including conjunctival hyperemia, chemosis, epiphora, or increased secretions. Patients with ocular symptoms were more likely to have higher white blood cell and neutrophil counts and higher levels of procalcitonin, C-reactive protein, and lactate dehydrogenase than patients without ocular symptoms [3]. Feng et al. reported in 2021 that 9.5% of hospitalized COVID-19 patients exhibited ocular signs and symptoms, whereas Chen et al. found that 5.0% of their 535 studied patients presented with conjunctival congestion as the initial COVID-19 symptom [4]. In the present study 8.25% of the hospitalized patients were positive for conjunctival COVID-19 swab PCR test.

On the basis of previous experimental [29, 30] and human [31] studies one can consider a secondary conjunctival infection due to spreading of infection along mucus membranes. Also, human studies have shown that ACE2 and other SARS-CoV-2 co-receptors are expressed in human ocular tissues [31] and that certain diseases can change protein expressions. This may be important, since our study demonstrated that ocular symptoms were present in patients whom had a history of ophthalmologic diseases, which can potentially change the expression of viral receptor proteins [31].

In hospital settings SARS-CoV-2 can be also transmitted by virus carrying stuff or patients derived respiratory droplets [32] reaching the conjunctiva of patients. In addition, SARS-CoV-2 RNA has been detected post mortem in conjunctival tissues and intravitreal biopsies [33]. Thus the original entry of virus cannot always be ascertained.

All in all, the previous and present findings suggest that the conjunctiva can be seriously affected by COVID-19, either directly by the virus or indirectly from systemic COVID diseases. Also, due to the special anatomical connection between the conjunctiva and nasal cavities, the ocular surface can be an entry route of nasopharyngeal COVID-19 infection, as the tear film is transported to the inferior nasal meatus by the nasolacrimal duct [8].

Limitation of the study

There are limitations of our pilot study. In the COVID Care Centers of Semmelweis University detailed ophthalmic assessment was not possible due to the personal protective equipment including compulsory face shield. Thus, we focused the investigation on laboratory parameters and the ocular anterior segment symptoms. In addition, we could investigate only limited number of cases, and no technical replicates were done. During the time of our investigation in early 2021, only limited amount of COVID vaccines was available in Hungary, mostly distributed among healthcare workers and elderly patients above the age of 80 years. Thus, the patients included in this study received none or only one dose of one of the COVID vaccines, mostly Pfizer, Moderna, AstraZeneca, Sinopharm or Sputnik, and because of that we did not record immunization history.

CONCLUSIONS

Although only in few cases, the data of the present study provides a proof of concept that SARS-CoV-2 can be present on the conjunctival mucosa even in nasopharyngeal negative patients, a finding, which can have clinical importance. Also, on the basis of this findings one can hypothesize that – in addition to the respiratory tract – the conjunctiva can be an entrance route for SARS-CoV-2 to the human body, which however needs to be substantiated by follow up studies. Nevertheless, in high risk conditions in addition to covering the mouth and nose with mask, the protection of the eyes is also strongly recommended.

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