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Occurrence of SARS-CoV-2 in the intraocular milieu

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ABSTRACT

The purpose of this research is to study the intraocular occurrence of SARS-CoV-2.

In postmortem examinations, aqueous humor and the vitreous samples were collected. All individuals were previously positive in nasopharyngeal swabbing and cause of death was respiratory failure due to SARS-CoV-2 infection. Testing was done using quantitative RT-PCR.

We included 16 aqueous humor and 16 vitreous samples for PCR testing. None of the results was positive for SARS-CoV-2. Human GAPDH genes to verify the presence of RNA was present in all aqueous humor samples (16/16, 100%) and 15/16 (93.8%) vitreous samples.

In conclusion, this case series found no evidence of SARS-CoV-2 in the intraocular milieu.

In December 2019, a new corona virus was found causing pneumonia in Wuhan, Hubei Province, China. Within only weeks, this novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), started spreading worldwide. On March 11th, 2020, it was recognized as a pandemic by the World Health Organization (WHO) (WHO on Covid-19, website).

To date, it is known that SARS-CoV-2, transmitted via aerosols, binds to angiotensin-converting-enzyme-2 (ACE2) to enter host cells (Wan et al., 2020). Direct contact with mucous membranes is suspected to be the route of transmission. Besides the respiratory system, the ocular surface is known to be involved (Lu et al., 2020; Zhang et al., 2020). Pathways of infections described so far are inoculation of the ocular tissues from respiratory droplets or aerosolized virus particles, migration from the nasopharynx through the nasolacrimal duct and hematogenous spread through the lacrimal gland (Seah and Agrawal, 2020).

Up to date, ocular occurrence was described to be limited to the conjunctiva mainly and retinal changes have been described. However, ocular manifestation may be asymptomatic and develop after some time (Chen et al., 2020; Siedlecki et al., 2020; Wu et al., 2020; Xia et al., 2020; Zhang et al., 2020). In this study, we tested for SARS-CoV-2 in the aqueous humor and the vitreous representing the intraocular milieu.

In sixteen individuals with confirmed SARS-CoV-2 infection samples from the vitreous and aqueous humor were taken during postmortem examinations. All included individuals had to be positive for the virus in nasopharyngeal swabbing. No exclusion criteria were defined. Causes of deaths were determined at autopsies. Medical histories were surveyed for nasopharyngeal swabbing results. Ophthalmic records were reviewed for all patients. None of the patients had a vitrectomy in the past history. Two eyes were pseudophakic (cataract surgery in 2016 and 2017 respectively), one eye was amblyopic, one eye received excimer laser and one eye received four intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections for diabetic macular edema, last injection in March 2020.

Positive vote was obtained by the ethics committee from the Medical University of Graz (EC number: 32–349 ex 19/20). The study adhered to the tenets of the Declaration of Helsinki.

Samples were taken at the absolute beginning of the postmortem examination from aqueous humor using 28-gauge needles and vitreous using 27-gauge needles. At first, a needle was placed in the anterior chamber and approximately 1–1.5 ml of clear liquid was obtained. Second, another sterile needle and syringe was used to extract vitreous fluid. 3–5 ml of a clear, slightly brownish liquid were aspirated. Only right eyes were included.

During the postmortem examination swabs were taken from the nose, pharynx, trachea and lung and tissue samples were taken from the gastrointestinal tract and the respiratory tract in 15 patients. 10/15 (66.7%) swabs and 10/15 (66.7%) tissue samples were positive for SARS-CoV-2.

qRT-PCR was performed using a RdRP gene assay and probe specific to SARS-CoV-2 (Corman et al., 2020). Briefly, primers, probes and 5 μl of

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Table 1
Characteristics and demographics.

Pat. ID	Sex	Age	Positiv for (in days)	Last positiv before death (in days)	Positive swabs	Time until autopsy (in hours)	Aqueous humor	GAPDH aqueous humor	Vitreous body	GAPDH vitreous body	Cause of death at autopsy
1	F	71	5	5	1	30	N/A	34,4	N/A	N/A	Diffuse alveolar damage
2	F	93	9	9	1	20	N/A	31,6	N/A	29,37	Diffuse alveolar damage
3	M	79	13	5	2	25	N/A	32,25	N/A	30,48	Diffuse alveolar damage
4	F	92	9	7	2	24	N/A	33,67	N/A	32,02	Diffuse alveolar damage
5	M	85	3	2	2	5	N/A	31,77	N/A	33,56	Acute (aspiration) pneumonia
6	M	77	9	1	4	53	N/A	28,9	N/A	22,91	Diffuse alveolar damage
7	M	71	7	7	1	50	N/A	26,08	N/A	26,34	Diffuse alveolar damage
8	M	77	6	6	1	19	N/A	30,9	N/A	31,53	Acute (necrotizing) pneumonia
9	M	85	10	3	2	13	N/A	30,58	N/A	23,98	Diffuse alveolar damage
10	M	67	20	0	2	22	N/A	27,86	N/A	29,4	Diffuse alveolar damage
11	F	80	7	4	3	45	N/A	27,57	N/A	27,67	Severe acute pneumonia
12	F	80	11	5	3	21	N/A	28,18	N/A	34,19	Acute (necrotizing) pneumonia
13	M	80	6	2	3	4	N/A	31,86	N/A	25,91	Severe acute pneumonia
14	F	79	2	2	1	10	N/A	23,14	N/A	34,69	Diffuse alveolar damage
15	M	53	23	2	5	13	N/A	31,1	N/A	29,39	Diffuse alveolar damage
16	M	65	34	8	4	32	N/A	32,34	N/A	29,2	Severe acute pneumonia

Abbreviations: F: female, GAPDH: glycerinaldehyde-3-phosphate dehydrogenase, M: male, N/A: no answer, Pat. ID: patient identification.

RNA were added to 10 μl of SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (Thermo Fisher) mastermix. PCR was performed on a Quantstudio 7 instrument (Thermo Fisher) with the following cycling conditions: 55 °C 15min, 95 °C 3min; 45 cycles (95 °C 15sec; 58 °C 30sec). Amplification data was downloaded and processed using the qpcR package of the R project (https://www.r-project.org/). Amplification efficiency plots were visually inspected and Cp2D (cycle peak of second derivative) values were calculated for samples with valid amplification curves. Plots were generated with R using the reshape, tidyverse and ggplot packages.

To exclude the possibility of PCR inhibition and to ascertain the presence of extracted RNA in each diagnostic sample, we additionally performed a qRT-PCR assay for the human GAPDH gene. Primers used for this assay were GAPDH-fwd 5′-CCTCCACCTTTGACGCT, GAPDH-rev 5′-TTGCTGTAGCCAAATTCGTT and GAPDP-probe FAM-5′-AGCTTGACAAAGTGGTCGTTGAGGGCAATG. These were present in all aqueous humor samples (16/16, 100%) and 15/16 (93.8%) vitreous samples.

We included aqueous humor and vitreous samples from 16 individuals during postmortem examinations. The median age at death for the study individuals was 79 (53–93) years. 6/16 (37.5%) of patients were female. Study individual characteristics are summarized in Table 1.

PCR was negative for all aqueous humor and vitreous samples for SARS-CoV-2.

Causes of death at autopsies were diffuse alveolar damage in 10/16 (62.5%) and acute pneumonia in 6/16 (37.5%). The median time between death and autopsy was 21.5 (4–53) hours.

Patients had a median of 2 (1–5) positive nasopharyngeal swabs for median 9 (2–34) days. The last positive swabs were performed in median 4.5 (0–9) days before death (Table 1).

In this case series, SARS-CoV-2 could not be found in the aqueous humor and in the vitreous. To the best of our knowledge, this is the first study investigating on the intraocular occurrence of SARS-CoV-2.

The presence of SARS-CoV-2 has previously been described on the ocular surface, including the tears, conjunctiva and nasolacrimal duct (Chen et al., 2020; Seah and Agrawal, 2020; Wu et al., 2020; Xia et al., 2020; Zhang et al., 2020). We also know that ACE2 is expressed on mucosal membranes such as the conjunctiva, but also inside the bulbus, mainly located in the posterior structures, such as the iris, ciliary body and retina (ganglion cells, inner nuclear layer cells, photoreceptor cells, endothelial cells from the retina and choroidea and retinal pigment epithelium), making it a potential target for the virus (Holappa et al., 2017; Qing et al., 2020; Savaskan et al., 2004; Senanayake et al., 2007).

Although SARS-CoV-2 primarily affects the lungs, there are reports on occurrence in the central nervous system. In two cases SARS-CoV-2 was detected in cerebrospinal fluid (CSF), two cases found evidence for encephalitis with and without positive evidence in CSF (Moriguchi et al., 2020; Poyiadji et al., 2020). Although the mechanisms of infections are not yet fully understood, a breakdown of the blood-brain-barrier due to cytokine storm was proposed (Poyiadji et al., 2020). Unfortunately, none of those reports considered intraocular occurrence, linking the breakdown of the blood-brain-barrier to a possible breakdown of the blood-retina-barrier. In these patients, SARS-CoV-2 might be found in the aqueous humor and/or vitreous. None of our patients were positive in the CSF.

Therefore, our data cannot exclude the possibility, that patients with central nervous system involvement may also have intraocular occurrence. Furthermore, our sample size is quite small and thus, intraocular involvement cannot be excluded totally.

Just recently, Marinho et al. described retinal changes in optical coherence tomographies (OCT) in 11 patients positive to SARS-CoV-2 (Marinho et al., 2020). All patients showed hyper-reflective lesions at the level of ganglion cell and inner plexiform layers in both eyes and four patients showed subtle cotton wool spots and microhemorrhages along

the retinal arcade. However, visual acuity remained normal and none of the patients showed signs of intraocular inflammation. These retinal findings support our hypothesis that SARS-CoV-2 might be able to occur in the intraocular milieu.

The impact of time delay between time of death and sample taking on detectability of SARS-CoV-2 remains unknown, especially in the intraocular milieu. In a study by Chin et al. it was shown that SARS-CoV-2 was still detectable after 14 days at +4 °C and after 7 days at +22 °C in a virus transport medium (Chin et al., 2020). We therefore assume that the time gap between death and autopsy had no impact on the detectability of the virus. None of the eyes had a vitrectomy.

In subsequent studies, it would be interesting whether the intraocular milieu, an immune-privileged environment, pools antibodies and whether there is a linkage between the positive results in the CSF/brain and the intraocular milieu.

In conclusion, this case series did not find SARS-CoV-2 in aqueous humor and vitreous samples during postmortem examinations of 16 patients with cause of death by SARS-CoV-2 infections.

Declarations of interest

None.

Author contributions

Wolfgang List and Laura Posch-Pertl reviewed the literature, formed the concept of this study and obtained the approval from the Ethics Committee of the Medical University of Graz. Wolfgang List drafted the manuscript. Andreas Wedrich, Laura Posch-Pertl and Wolfgang List interpreted the data and discussed and revised the manuscript. Peter Regitnig, Karl Kashofer, Gregor Gorkiewicz and Martin Zacharias performed the post-mortem examinations, collected the samples and analyzed and interpreted the data.

Data accessibility

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Data was available to all authors.

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References

Chen, L., Liu, M., Zhang, Z., Qiao, K., Huang, T., Chen, M., Xin, N., Huang, Z., Liu, L., Zhang, G., Wang, J., 2020. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. Br. J. Ophthalmol. 104 (6), 748–751.

Chin, A.W.H., Chu, J.T.S., Perera, M.R.A., Hui, K.P.Y., Yen, H.L., Chan, M.C.W., Peiris, M., Poon, L.L.M., 2020. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 1, e10.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T., Brunink, S., Schneider, J., Schmidt, M.L., Mulders, D.G., Haagmans, B.L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.L., Ellis, J., Zambon, M., Peiris, M., Goossens, H., Reusken, C., Koopmans, M.P., Drosten, C., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 25.

Holappa, M., Vapaatalo, H., Vaajanen, A., 2017. Many faces of renin-angiotensin system - focus on eye. Open Ophthalmol. J. 11, 122–142.

Lu, C.W., Liu, X.F., Jia, Z.F., 2020. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet 395, e39.

Marinho, P.M., Marcos, A.A.A., Romano, A.C., Nascimento, H., Belfort Jr., R., 2020. Retinal findings in patients with COVID-19. Lancet 395, 1610.

Moriguchi, T., Harii, N., Goto, J., Harada, D., Sugawara, H., Takamino, J., Ueno, M., Sakata, H., Kondo, K., Myose, N., Nakao, A., Takeda, M., Haro, H., Inoue, O., Suzuki-Inoue, K., Kubokawa, K., Ogihara, S., Sasaki, T., Kinouchi, H., Kojin, H., Ito, M., Onishi, H., Shimizu, T., Sasaki, Y., Enomoto, N., Ishihara, H., Furuya, S., Yamamoto, T., Shimada, S., 2020. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int. J. Infect. Dis. 94, 55–58.

Poyiadji, N., Shahin, G., Noujaim, D., Stone, M., Patel, S., Griffith, B., 2020. COVID-19-associated acute hemorrhagic necrotizing encephalopathy: CT and MRI features. Radiology, 201187.

Qing, H., Li, Z., Yang, Z., Shi, M., Huang, Z., Song, J., Song, Z., May 2020. The possibility of COVID-19 transmission from eye to nose. Acta Ophthalmol. 98 (3), e388.

Savaskan, E., Loffler, K.U., Meier, F., Muller-Spahn, F., Flammer, J., Meyer, P., 2004. Immunohistochemical localization of angiotensin-converting enzyme, angiotensin II and AT1 receptor in human ocular tissues. Ophthalmic Res. 36, 312–320.

Seah, I., Agrawal, R., 2020. Can the coronavirus disease 2019 (COVID-19) affect the eyes? A review of coronaviruses and ocular implications in humans and animals. Ocul. Immunol. Inflamm. 28, 391–395.

Senanayake, P., Drazba, J., Shadrach, K., Milsted, A., Rungger-Brandle, E., Nishiyama, K., Miura, S., Karnik, S., Sears, J.E., Hollyfield, J.G., 2007. Angiotensin II and its receptor subtypes in the human retina. Invest. Ophthalmol. Vis. Sci. 48, 3301–3311.

Siedlecki, J., Brantl, V., Schworm, B., Mayer, W.J., Gerhardt, M., Michalakis, S., Kreutzer, T., Priglinger, S., May 2020. COVID-19: ophthalmological aspects of the SARS-CoV 2 global pandemic. Klin Monbl Augenheilkd 237 (5), 675–680.

Wan, Y., Shang, J., Graham, R., Baric, R.S., Li, F., 2020. Receptor recognition by the novel coronavirus from wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J. Virol. 94.

Wu, P., Duan, F., Luo, C., Liu, Q., Qu, X., Liang, L., Wu, K., May 1 2020. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol 138 (5), 575–578.

Xia, J., Tong, J., Liu, M., Shen, Y., Guo, D., May June 2020. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J. Med. Virol. 92 (6), 589–594.

Zhang, X., Chen, X., Chen, L., Deng, C., Zou, X., Liu, W., Yu, H., Chen, B., Sun, X., July 2020. The evidence of SARS-CoV-2 infection on ocular surface. Ocul. Surf. 18 (3), 360–362.

Webreference

World Health Organization Who, 2020. On the breakout of Corona-Virus (Covid-19). https://www.euro.who.int/de/health-topics/health-emergencies/coronavirus-covid-19/novel-coronavirus-2019-ncov/. (Accessed 2 July 2020).