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Evaluation of the presence of SARS-CoV-2 in the aqueous humor and vitreous in patients undergoing combined phaco-vitrectomy surgery

Kombine fako-vitrektomi ameliyatı geçiren hastalarda aköz hümör ve vitreusta SARS-CoV-2 varlığının değerlendirilmesi

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Abstract

Purpose: To investigate the presence of virus in the aqueous humor and vitreous of patients undergoing elective combined cataract and pars plana vitrectomy during the Coronavirus disease 2019 (COVID-19) pandemic.

Materials and methods: In this prospective cross-sectional study, of the patients to undergo elective surgery, those who had a negative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nasal swab test 24-72 hours prior to the surgery and who were asymptomatic were included. SARS-CoV-2 IgG antibodies were evaluated in blood samples taken 24-72 hours before the operation. 0.1 cc of aqueous humor and 0.1 cc of vitreous fluid were aspirated at the beginning of the surgery. The presence of SARS-CoV-2 viral ribonucleic acid (RNA) was evaluated by real-time reverse transcriptase polymerase chain reaction (RT-PCR).

Results: Of 66 participants, 39 were male (59.1%) while 27 were female (40.9%). Twenty-five patients (37.8%) had a history of COVID-19 between 20 days-60 days (mean 49 days) before the surgery. There were 58 patients (87.9%) with a history of vaccination before the operation and 8 patients (12.1%) without a history of vaccination. No SARS CoV 2 RNA was detected in the aqueous humor and vitreous samples of any patient. IgG against SARSCoV-2 was detected in 3 patients who had not been vaccinated against COVID-19 before and had no known history of COVID-19.

Conclusion: We did not find any SARS-CoV-2 viral genetic material in the aqueous and vitreous fluids of asymptomatic participants whose nasal swab test results were negative, even if they recently had COVID-19. There is a need for more comprehensive studies investigating the latency of the virus in immune-privileged areas such as the eye, how long it remains in the eye even if it is withdrawn from the circulation, and possible eye diseases that it may cause during the convalescence period.

Key words: Aqueous humor, COVID-19, SARS-CoV-2, vitreous.

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Öz

Amaç: Coronavirus 2019 (COVID-19) pandemisi döneminde elektif kombine katarakt ve pars plana vitrektomi cerrahisi uygulanan hastaların aköz ve vitreus sıvısında virus varlığının araştırılması amaçlandı.

Gereç ve yöntem: Bu prospektif kesitsel çalışmaya elektif kombine katarakt ve pars plana vitrektomi ameliyatı yapılacak hastalardan preoperatif 24-72 saat önceki SARS-CoV-2 nazal sürüntü testi negatif çıkan, COVID-19 bakımından asemptomatik olanlar dahil edildi. Preoperatif 24-72 saat önce alınan kan numunelerinde SARS-CoV-2 IgG antikoru değerlendirildi. Katarakt cerrahisinin başlangıcında 0.1 cc aköz hümör, vitrektominin başlangıcında ise 0.1 cc vitreus sıvısı aspire edildi. SARS-CoV-2 viral ribonucleic acid (RNA) varlığı real-time reverse transcriptase polymerase chain reaction (RT-PCR) ile değerlendirildi.

Bulgular: Otuz dokuzu erkek (%59,1), 27'si kadın (%40,9) olmak üzere 66 katılımcının göz içi sıvı örnekleri başarıyla analiz edildi. 25 hastada (%37,8) operasyondan 20 gün-60 gün önce (ortalama 49 gün) COVID-19 geçirme öyküsü mevcuttu. Operasyondan önce aşı öyküsü bulunan 58 hasta (%87,9), aşı öyküsü bulunmayan 8 hasta (%12,1) mevcut idi. Hiçbir hastanın aköz hümör ve vitreus örneklerinde SARS-CoV-2 RNA'sına rastlanmadı. Daha önce COVID-19 aşısı yapılmamış ve bilinen COVID-19 geçirme öyküsü olmayan 3 hastada SARSCoV-2'ye karşı gelişen IgG tespit edildi.

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Sonuç: Bu çalışmada COVID-19 bakımından asemptomatik, nazal sürüntü testi negatif katılımcıların aköz ve vitreus sıvılarında SARS-CoV-2 viral genetik materyaline rastlamadık. Virüsün göz gibi immün ayrıcalıklı alanlarda latent kalma durumunu, dolaşımdan çekilse bile gözde ne kadar süre kaldığını ve nekahat döneminde neden olabileceği olası göz hastalıklarını araştıran daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Aköz hümör, COVID-19, SARS-CoV-2, vitreus.

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Introduction

Coronavirus disease 2019 (COVID-19), first reported in December 2019 in Wuhan, China's Hubei province, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. It was declared a global pandemic by the World Health Organization (WHO) on March 11, 2020. The virus can lead to clinical pictures ranging from asymptomatic infection to serious illness and death. The respiratory system is the most frequently affected system, but the virus shows neurotropism and endothelial tropism, causing a cytokine storm by creating a general inflammatory reaction [1, 2]. Conjunctivitis and keratoconjunctivitis are the most common ocular symptoms. Conjunctival irritation is the most common ophthalmologic finding (50.8%), followed by diplopia (27.8%) and cotton wool patches (27.8%) [3].

As of 6 January 2023, 657.977.736 cases of COVID-19 were reported to WHO, including 6.681.433 deaths. As of 21 December 2022, a total of 13.073.712.554 vaccine doses have been administered. Accordingly, it is thought that approximately 15 million people have died in the last two years, either due to the coronavirus or the negative effect of the virus on systems. In studies conducted during that period, it was emphasized that the different modes of spread of SARS-CoV-2, systemic organ involvement, and the possibility of the viral reservoir in humans should be comprehensively investigated.

The eye is a recognized immune-privileged location that harbors viruses. Previously, Varkey et al. [4] and Gonzales et al. [5] detected live Ebola and Rubella viruses in the aqueous humor, respectively. Considering the high number of cases in ophthalmology practice and the physical close contact of the ophthalmologist with the patient during eye examination and interventions, the presence of SARS-CoV-2 in the eye has also been investigated in various studies in order to take measures to reduce pathogen contamination. Most of these studies have been conducted either with postmortem tissues from people who died due to the disease or on a limited number of patients with COVID-19. Koo et al. [6], on the other hand, demonstrated the presence of the virus for the first time in the aqueous fluid of elective anterior segment surgery patients who were asymptomatic for COVID-19 and had a negative SARS-CoV-2 nasal swab test. This suggests that the virus can stay in asymptomatic individuals beyond the blood-ocular barrier, raising the possibility that it could persist in immune-privileged environments despite the lack of symptoms [3, 6]. To date, many studies have been conducted on SARS-CoV-2 in aqueous humor and vitreous samples. These were done on a small number of case samples, usually in patients known to have COVID-19. Although it is seen that the COVID-19 pandemic has started to regress and the number of deaths is rapidly decreasing, no studies investigating the simultaneous presence of the virus in both the anterior chamber and vitreous in asymptomatic individuals with negative nasal swab test have not been conducted to date.

In this study, we aimed to investigate the presence of SARS-CoV-2 viral material in both aqueous humor and vitreous samples in elective surgery patients and to evaluate their relationship with serum immunity level.

Materials and methods

This prospective cross sectional study was conducted from Dec 2021 to March 2022 in Adana City Training and Research Hospital which is a tertiary hospital attached to a COVID-19 facility. The Institutional Research and Ethics Committee granted ethical clearance for the study (95/1686), which was carried out in accordance with the Declaration of Helsinki's principles. Informed consent was obtained from

all individual participants included in the study.

Patients who were planned to undergo elective combined phaco-vitrectomy surgery, over 18 years of age, of both genders, who gave consent to participate in the study, whose data were fully accessible, and whose nasal swab was negative for SARS-CoV-2 real-time reverse transcriptase polymerase chain reaction (RT-PCR) Test performed 24-72 hours before the operation were included in the study. Those who did not consent to participate, under the age of 18, with possible COVID-19 symptoms such as fever, cough, absence of smell and taste immediately before surgery, with positive SARS-CoV-2 PCR test, with incomplete data, and from whom sufficient volume of aqueous or vitreous samples could not be obtained were excluded from the study.

The COVID-19 dates, the vaccination dates, and the names of the vaccines were recorded from the patients' electronic files. The operations were performed by the same senior surgeon (E.Ç.) under topical or general anesthesia. Using a 1-cc syringe and a 30-G cannula, approximately 0.1 cc of aqueous humor was collected at the beginning of combined phacoemulsification- pars plana vitrectomy. At the beginning of the vitrectomy, 0.1 cc of vitreous was aspirated from the 25-G trocar inlet 4 mm behind the pars plana, before the infusion was opened with the help of a cannula.

Viral RNA was extracted with the QIAamp Viral RNA Mini kit Cat: 52906 (QIAGEN) according to the protocols. After viral RNA extraction, RNA quality was assured via nanodrop measurement, determined using Thermo Scientific ND8000 Uv/vis spectrophotometer [7]. The viral RNA amplification was performed using the One Step PrimeScript III RT-qPCR Kit (Takara). All reactions were conducted ~~out~~ on a CFX96 Touch instrument with the following Real-Time-PCR conditions: 52°C for 5 min, 95°C for 10 sec, followed by 44 cycles at 95°C for 5 sec and 55°C for 30 sec. The primer and probe sequences used for RT-PCR are targeted against the Nucleocapsid (NC) gene of SARS-CoV-2 with the following primers and probes: N1 Forward: 5'-GAC CCC AAA ATC AGC GAA AT-3', N1 Reverse: 5'-TCT GGT TAC TGC CAG TTG AAT CTG-3' N1 Probe: 5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3 N2 Forward: 5'-TTA CAA ACA TTG GCC GCA AA-3' N2 Revers: 5'-GCG CGA CAT TCC GAA GAA-3' N2 Probe: 5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3 [7, 8]. For inhibition control, another primer and probe set targeted against the human RNase P gene was used as: RP-F RNase P Forward Primer AGA TTT GGA CCT GCG AGC G, RP-R RNase P Reverse Primer GAG CGG CTG TCT CCA CAA GT, RP-P RNase P Probe FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1.

In the samples, the IDT nCoV-N Positive Control (1/10 diluted) included in the CDC Primer kit gave a CT value between 25-27. On the other hand, human samples have the same result as the negative control used in the experiment. The positive and negative control in the experiment worked, so the experiment worked.

IgG developed against SARS-CoV-2 was examined in serum samples taken 24-72 hours before the operation. The binding activity of antibodies in the serum of each patient against SARS-CoV2 Spike Protein receptor binding domain (RBD) was measured with an in-house indirect ELISA test. Briefly, 50 ng of a purified recombinant protein RBD were coated into a 96-well ELISA plate (nuncMaxisorp) overnight at 4°C. Wells were blocked with milk powder in PBS for 1 hour at 37°C, followed by incubation with diluted antisera and positive and negative control serum for 1 hour at 37°C. A diluted horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG antibody was added for 1 hour at room temperature. Wells were washed three times between each step and five times for the last step with Tween-20 buffer in PBS. Wells were developed using 3,3,5,5-tetramethylbenzidine (TMB) and read at 450 nm after terminated with 2M H_2SO_4.

Statistical analysis

We used IBM SPSS statistics version 26 for Windows to analyse the data obtained from the study. Numerical data were given as mean, minimum, and maximum while categorical data were expressed as numbers (n) and percentages (%).

Results

Of the 66 patients included in the study, 39 (59.1%) were male and 27 (40.9%) were female, with a mean age of 60.1±9.14 (28-75) years.

Tractional retinal detachment was present in 36 patients, vitreous hemorrhage in 17 patients, epiretinal membrane in 9 patients, macular hole in 4 patients, and cataract in all patients. 25 patients (37.8%) had a history of COVID-19 between 20 days and 60 days (mean 49 days) before the operation. Eight patients who had never been vaccinated before the operation, 3 patients with a single dose, 22 patients with 2 doses, 20 patients with 3 doses, and 13 patients with 4 doses. It was known that six patients had previously been vaccinated with Sinovac (CoronaVac), 31 patients with Pfizer–BioNTech, and 21 patients with both Sinovac (CoronaVac) and Pfizer–BioNTech. The last vaccine dose of 58 patients (87.9%) who had a history of vaccination before the surgery was administered an average of 90 days (7-270 days) before the surgery. The flow chart showing the immune status of 8 patients (12.1%) who had no previous vaccination history is given in Figure 1.

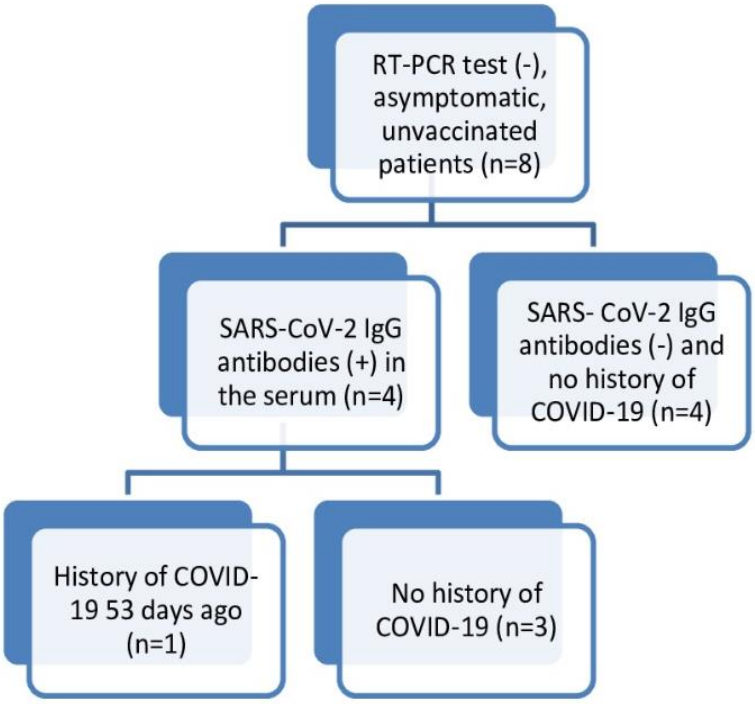

Figure 1. Flowchart demonstrating the immune status of unvaccinated patients

Fifty patients were operated on under topical anesthesia whereas 16 patients were operated on under general anesthesia. The mean surgery duration was 105 minutes (90-125 minutes). Intraocular fluid samples taken from the right eye of 37 patients and the left eye of 29 patients were analyzed. No SARS-CoV-2 RNA was detected in the aqueous humor and vitreous samples of any patient. No COVID-19 symptoms were observed in any of the patients in the 72-hour period after the operation. No ocular complications were encountered in the postoperative follow-up of the patients. IgG against SARS-CoV-2 was detected in the serum of 3 patients who had not been vaccinated against COVID-19 before and had no known history of COVID-19 disease.

Discussion

This study was conducted to determine the presence of SARS-CoV-2 in the aqueous humor and vitreous in patients undergoing combined phaco-vitrectomy surgery. We did not find any SARS-CoV-2 viral genetic material in asymptomatic participants whose nasal swab test was negative, even if they recently had COVID-19.

During the COVID-19 pandemic, outpatient and inpatient surgeries were limited and the number of ophthalmologic cases fell sharply. With the decrease in the number of COVID-19 cases and the abandonment of the pandemic rules, the number of ophthalmic surgeries has increased rapidly. In this study, SARS-

CoV-2 viral genetic material in aqueous humor and vitreous samples and SARS-CoV-2 IgG antibody levels in the serum of asymptomatic individuals with negative nasal swab test who presented for elective combined phaco and pars plana vitrectomy surgery were evaluated.

Ocular symptoms and signs are frequently encountered in COVID-19 patients. In these cases, either the haematogenous route to the posterior segment or direct implantation of viral load in conjunctival mucosa can result in ocular symptoms [9, 10]. Sometimes ocular symptoms may precede respiratory or other symptoms. Adults most frequently experience follicular conjunctivitis, conjunctival hyperemia, chemosis, and epiphora. Patients with COVID-19 have been documented to develop uveitis, optic neuritis, disc edema, retinal artery occlusions, intraretinal hemorrhages and cotton wool spots. Signs and symptoms of neuro-ophthalmology include myasthenia gravis, diplopia, and ocular discomfort. Whether these symptoms result from direct infection of the eye, immunologic reactions, or ischemic damage to the visual system is not clear [11].

The presence of SARS-CoV-2 has previously been described on the ocular surface, including the tears, conjunctiva and nasolacrimal duct [12, 13]. Studies investigating the presence of SARS-CoV-2 genetic material or proteins in intraocular fluids or tissues have generally been conducted on people known to be infected with the virus or on postmortem tissues. In the first study evaluating the presence of the intraocular virus, the SARS-CoV-2 PCR test was found to be negative in postmortem aqueous humor and vitreous samples of 16 patients whose nasopharyngeal swab tests were positive and the cause of death was respiratory failure due to SARS-CoV-2. It was considered that the amount of time between a person's passing and their autopsy had no bearing on the virus' detectability because SARS-CoV-2 was still detectable after 14 days at +4°C and 7 days at +22°C in a virus transit environment [14], and it was argued that due to the small sample size, intraocular involvement cannot be completely excluded with these results [15].

In another study conducted in 10 eyes of 5 patients who died from COVID-19, SARS-CoV-2-RNA was not detected in any of the human vitreous and retinal samples. Histopathological examinations revealed no signs of viral damage to the retinal vasculature or tissues. It was emphasized that SARS-CoV-2 infection of human retinal tissue and/or vitreous fluid, if viral replication is possible, is extremely rare in COVID-19 postmortem donors, therefore retinal histopathology was normal in a small patient cohort. In addition, negative results have been explained by the view that pathological changes may not be permanent in individuals succumbing to COVID-19 [16]. In a larger study evaluating 28 conjunctiva, 30 aqueous humor, and 30 vitreous fluid, SARS-CoV-2 RNA was evaluated in postmortem ocular specimens, and positive results were found in 1 conjunctival and 2 vitreous specimens in 3 different patients [17]. Compared to the aforementioned studies, the higher number of samples in this study may have resulted in positive results.

Sanjay et al. [18] isolated SARS-CoV-2 in the vitreous sample of a case with endogenous endophthalmitis and conducted the first study demonstrating the presence of intraocular virus in a live case. In another study, 7 posttraumatic patients with positive nasal swab test who were asymptomatic or had moderate COVID-19 were evaluated, and RT-PCR tests were found to be negative in 7 aqueous fluid samples and 5 vitreous fluid samples [19].

In the study conducted by Yan et al. [20], despite the date, 2 months after the reported infection, viral proteins were observed. The study revealed the possibility that SARS-CoV-2 proteins could be discovered inside of the eye and continue to exist even after the virus has appeared to have cleared up in the bloodstream. However, in our study, no genetic material was found in the intraocular fluids of 25 patients who were known to have had COVID-19 on average 49 days (20-60) ago, and in a patient who was unvaccinated but had SARS-CoV-2 IgG antibodies in the serum who was known to have had COVID-19 53 days ago. Additionally, there was no genetic material in the intraocular fluids of 3 patients who were unvaccinated and had SARS-CoV-2 IgG antibodies in the blood and had no history of COVID-19, possibly asymptomatic.

Jin et al. [21] reported a case who had acute graft rejection and tested positive for SARS-CoV-2 by PCR 5 days after the onset of ocular symptoms. This suggests that aqueous humor

may play a role in corneal endothelial rejection in some patients infected with SARS-CoV-2. Based on this study, Dr. Koo et al. [6] conducted the first study investigating SARS-CoV-2 in the aqueous humors of living individuals with a negative nasal swab test. PCR results were positive in aqueous humor in 6 of 31 samples with no symptoms and a negative nasal swab test. Information about the immunity level and vaccination status of the patients is not available in this study. We think that this positivity rate is quite high for these patients with a negative COVID-19 swab test and no symptoms.

In our study, in which we evaluated both anterior chamber and vitreous samples with a larger patient population, we did not find any genetic material belonging to the virus in either the aqueous or vitreous in any of the participants. In our study, the COVID-19 histories of the patients were determined not only according to their statements but also according to electronic records. We also evaluated the serum Ig G levels of our patients and found that there were 3 patients developed immunity in the serum, even if they were unvaccinated and had no known history of COVID-19. We think that these patients had the infection asymptomatically and gained immunity. One immunized patient who was unvaccinated and had a history of COVID-19 was COVID-19 positive 53 days before the operation. Although it has been previously shown that the SARS-CoV-2 antigen is present in a patient with a history of COVID-19 30 days prior [20], we think that the time of infection of these 4 patients who were unvaccinated and had Ig in their blood, long before the operation date, may have negatively affected the possible presence of virus in the intraocular fluid samples.

The presence of SARS-CoV-2 in body fluids including tears has been predicted to be associated with viral load and disease severity, as indicated by the Ct values of naso-oropharyngeal RT-PCR [22]. Contrary to the previous studies, no virus was found in the vitreous and aqueous fluid samples of any of the patients, suggesting that this may be related to the viral load and the increasing rate of vaccination today. In addition, as a limitation of the study, it may not be appropriate to investigate the possibility of latent virus in the vaccinated participant group.

In conclusion, in this study, we did not detect SARS-CoV-2 viral genetic material in the aqueous and vitreous of asymptomatic patients with negative nasal swab test for COVID-19. There is a need for more comprehensive studies investigating the latency of the virus in immune-privileged areas such as the eye and how long it remains in the eye even if it is withdrawn from circulation. In addition, more comprehensive studies are needed to investigate possible eye diseases that may be caused in the convalescent phase.

Conflict of interest: The authors declare that they have no conflict of interest.

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Ethics committee approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Adana City Training and Research Hospital Ethics Committee (95/1686, 16.12.2021) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by G.H.N.S., E.C., N.U., H.P., E.A. The first draft of the manuscript was written by G.H.N.S. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.