Refractive and topographic corneal changes associated with increased inflammatory mediators in tears after COVID-19

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This is a rare case report of a sudden shift to myopic astigmatism and topography alterations in a patient who experienced mild coronavirus disease 2019 (COVID-19) symptoms. A complete ophthalmologic evaluation, slit-lamp examination, corneal topography, and tear sampling for biochemical analysis were performed. Elevated corneal K-values were found, indicating abnormal anterior corneal curvature, based on the asymmetric corneal steepening index (cone location and magnitude index) in a Galilei G6 study. Tear analysis demonstrated elevated levels of interleukin (IL)-1β, IL-6, IP-10, IL-8, interferon-β, and prometalloproteinase-9. Ophthalmologists should maintain a high level of suspicion when symptoms suggestive of corneal involvement arise in patients recently infected with severe acute respiratory syndrome coronavirus 2.

Key words: Coronavirus, irregular astigmatism, myopia, SARS-CoV-2, tear cytokines, tear pro-MMP-9

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A 48-year-old woman, vaccinated for coronavirus disease 2019 (COVID-19) with three doses of AstraZeneca ChAdOx1-S on June 1, July 28, and November 5, 2021, was diagnosed with COVID-19 on January 8, 2022, based on clinical and epidemiological criteria. She had close contact with her husband, who tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through a rapid test and developed acute disseminated and necrotizing encephalomyelitis. She experienced occasional dry cough, sore throat, nasal congestion, fatigue, and a high-grade fever lasting for 3 days. Considering the SARS-CoV-2 epidemiology in Córdoba, Argentina, the causal variant may have been Omicron, which was predominant in December 2021.[1]

She had no history of previous COVID-19 infection. She was healthy, except for a prophylactic splenectomy that she underwent in 2008 due to a splenic artery aneurysm. No personal or family history of diseases was recorded, other than stable myopia since adolescence without any ocular surface manifestations or alterations. Her blood type was O RhD negative. Routine blood tests were unremarkable.

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Ten days after the diagnosis, the patient started experiencing acute blurred vision. Although no signs of conjunctivitis were found, she was prescribed topical ciprofloxacin 0.3% for 7 days by the emergency care department, which resulted in a slight improvement in her vision. A nasopharyngeal nucleic acid amplification test performed on January 21 came back negative for COVID-19. However, on February 1, a new episode of blurred vision occurred; the patient needed to be referred to ophthalmology department for complementary studies, and an increase in her baseline myopia was detected (from –2.25 D to –6.00 D), along with the appearance of astigmatism (4.75 D).

Her visual acuity was 20/30 in both eyes with fully corrected refraction using eyeglasses. Loteprednol and artificial tears were prescribed four times daily for 2 weeks, but no improvement was achieved. The patient reported nonspecific symptoms on the ocular surface, such as itching and burning, even with treatment, without clinical improvement. A mild keratitis was observed in the central corneal epithelium, along with conjunctival hyperemia. However, no significant tear film alterations were found in Schirmer's test, break-up time (BUT) test, or slit-lamp examination. Magnetic resonance imaging ruled out any optic nerve or brain lesions. Slit-lamp examination, fundoscopy, and retinal optical coherence tomography (OCT) showed normal findings.

The myopia worsened to –6.50 D within a week. Ophthalmic studies revealed bilateral moderate myopia with high astigmatism and an elevated corneal K-value greater than 49 D, suggesting asymmetric corneal steepening related to irregularity (cone location and magnitude index [CLMI]) based on the Galilei G6 analyzer report. OCT scans of the eye structures were performed to identify any additional abnormalities. Analysis of the corneal images showed an increase in epithelial thickness with marked central irregularity. However, the corneal stroma and posterior curvature power remained within normal values, consistent w ith the previous topographic analysis using the Galilei device [Fig. 1: pretreatment]. Therefore, the acute myopic astigmatism observed in the patient is likely due to the asymmetry of the anterior corneal curvature data associated with corneal

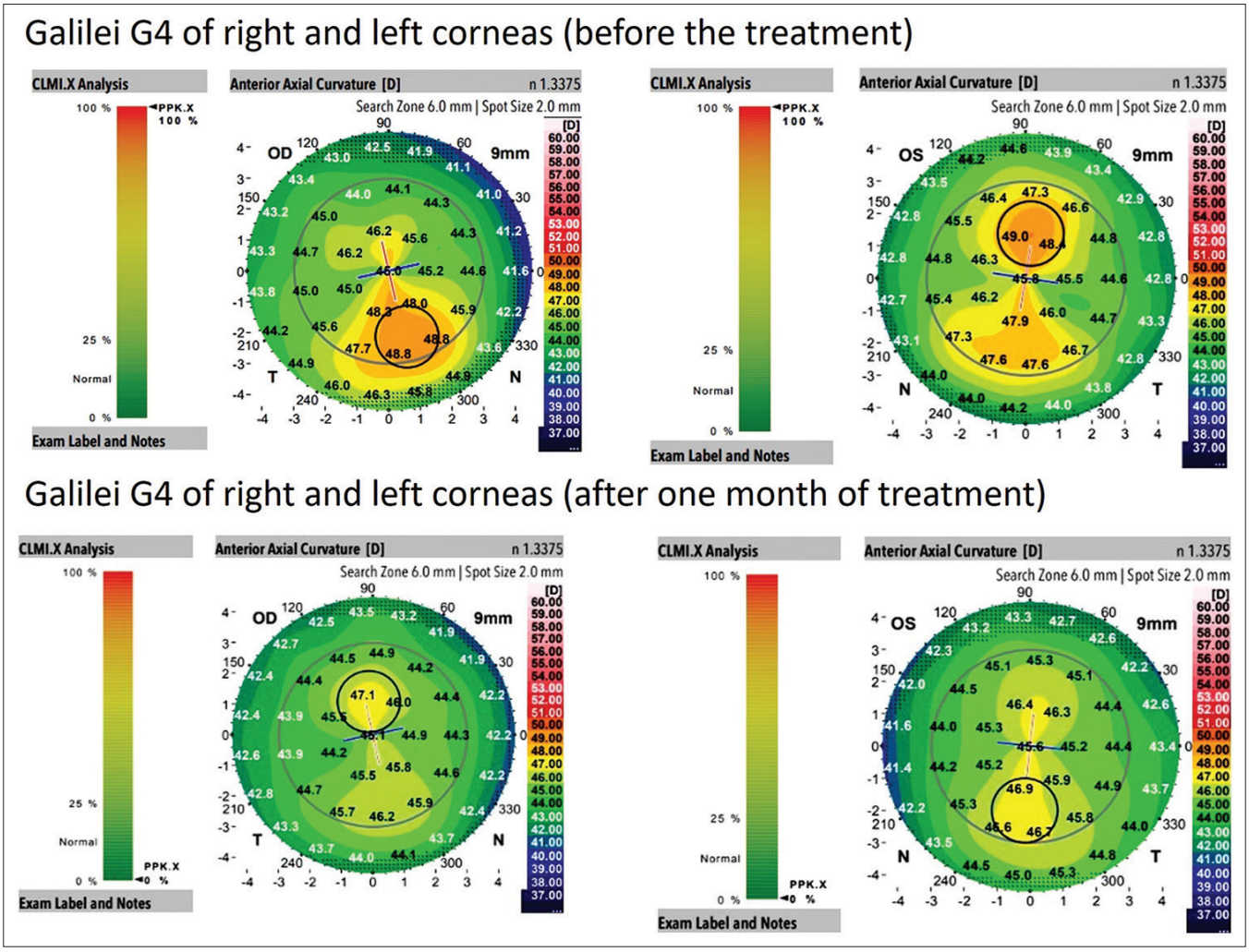

Figure 1: Galilei topographic analysis, specifically the cone location and magnitude index (CLMI), was performed before and after treatment with 0.1% topical cyclosporine. The analysis revealed a partial improvement in the ocular surface and corneal epithelium after 1 month of treatment. The current Galilei G6 computerized analysis has proven to be an excellent diagnostic tool with high precision and sensitivity for identifying irregular astigmatism associated with refractive abnormalities of the corneal surface. This allows for differentiation from typical ectasia. (Galilei dual Scheimpflug system, Ziemer Ophthalmic Systems AG, Port, Switzerland)

epithelium irregularity, rather than true ectasia, as suggested by the keratometric index analyzed by Galilei.

In addition, we examined the cross-sectional retinal and optic nerve structural images obtained from OCT and optical coherence tomography angiography (OCTA). The measurements taken from the retina, optic nerve head, retinal nerve fiber layer, and OCTA were all within normal range.

To investigate the concentrations of 13 cytokines and chemokines associated with inflammation and viral control in tears, including the patient's and those of two healthy controls (HCs), we utilized the LEGENDplex™ Human Anti-virus Response panel. In addition, the activity of metalloproteinases (MMPs) was determined using Gelatin sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zymography).

Elevated levels of interleukin (IL)-1β, IL-6, IL-8, Interferon gamma-induced protein 10 (IP-10), and interferon (IFN)-β were detected in tears from both eyes of the patient, with particularly higher levels observed in the fluid from the left eye [Table 1]. Prometalloproteinase-9 (Pro-MMP-9; 92 kDa) was present in all samples, but its expression was higher in the patient's tears. None of the tear samples showed detectable levels of IFN-λ1, IL-12p70, IFN-α2, IFN-λ2/3, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-10, IFN-γ, active MMP-9, pro-MMP-2, or MMP-2.

Following the diagnosis of increased levels of proinflammatory cytokines and MMPs in the patient's tear film, along with the presence of irregularities in the central corneal epithelium (contributing to the acute visual disturbance that had not responded to previous treatments), topical cyclosporine A (CyA) 0.1% sterile eye drops solution (Closporil®; Poen, Buenos Aires, Argentina) was initiated as a twice-daily treatment. The patient declined to undergo epithelial debridement for further histopathology and molecular biology studies.

After 1 month, the corneal irregularity observed in the topography analysis improved and returned to normal corneal examination values. Myopia decreased from −6 D to −3.75 D, and astigmatism decreased from 4 D to less than 1 D. However, the epithelial thickness (measured at 60 μm) still showed abnormal values [Fig. 1: after 1 month of treatment]. The levels of tear film cytokines and pro-MMP-9 after an additional 2 months of CyA treatment were significantly reduced compared to the levels measured before treatment. In fact, the concentrations were similar to or even lower than those found in tears from HCs [Table 1].

After her clinical condition became stable for 3 months, the patient decided to discontinue treatment with cyclosporine and artificial tears. One year later, no clinical (ocular surface symptoms) or topographic changes were observed. The patient maintained a myopia of −3.50 without astigmatism, and her best-corrected visual acuity was 20/20 in both eyes. A slight residual central epithelial thickening was still present in the Galilei analysis, but values for Schirmer's test and BUT were within the normal range. The patient described in this case report underwent strict follow-up for approximately 24 months from its initiation. It is noteworthy that the patient did not experience any other visual disturbances following the previously mentioned treatment.

Discussion

The COVID-19 pandemic has led to the emergence of several concerning variants.[2] The Omicron variant (B.1.1.529) is known for its increased transmissibility and ability to evade some vaccine protection, leading to a higher risk of reinfection. In addition, there is a possibility of increased or atypical disease severity with this variant.[3] In Argentina, the third wave of COVID-19, which began in December 2021, was predominantly associated with the Omicron variant.[1] While respiratory transmission is the primary mode of COVID-19 transmission, there is evidence to suggest that the virus can also affect the eyes and the ocular surface. Studies investigating ocular involvement in SARS-CoV-2 infection have reported not only ocular surface manifestations like conjunctivitis,[4] but also more severe conditions such as optic neuritis, uveitis, retinitis, and retinal vasculitis.[5-7]

Corneal tissue infection and its ophthalmologic implications have not been thoroughly studied yet. SARS-CoV-2 is believed to utilize angiotensin-converting enzyme 2 (ACE2) as a receptor to enter host cells. Several studies have examined the expression of ACE2 protein in various ocular tissues and have found that the corneal epithelium and superficial conjunctival cells express ACE2 and transmembrane serine protease 2 (TMPRSS2) receptors, which may make them susceptible to viral infection when compromised.[8] Ocular surface symptoms, particularly conjunctivitis, are commonly associated with direct viral infection of the conjunctiva. Most clinical research on SARS-CoV-2 and eye lesions has suggested that tears and the ocular surface could serve as potential routes of

Table 1: Quantification of cytokines and chemokines in tears using the LEGENDplex™ Human Anti-virus Response panel and detection of metalloproteinases using zymography

		IL-1β (pg/ml)	IL-6 (pg/ml)	IP-10 (pg/ml)	IL-8 (pg/ml)	IFN-β (pg/ml)	IFN-λ1, IL-12p70, IFN-α2, IFN-λ2/3, GM-CSF, IL-10, IFN-γ (pg/ml)	Pro-MMP9 Activity (relative units)	MMP-9, pro-MMP2, MMP2 Activity
Patient before treatment	LE	17.5	64.2	2734.4	1953.1	48.1	nd	45705 ± 214	nd
	RE	4.0	40.5	1976.0	589.8	28.0	nd	43258 ± 144	nd
Patient after treatment	LE	nd	nd	92.5	14.3	nd	nd	23359 ± 68	nd
	RE	nd	nd	157.2	39.4	nd	nd	23518 ± 38	nd
HC 1	LE	5.5	4.3	182.3	208.4	nd	nd	39439 ± 23	nd
HC 2	LE	nd	4.6	210.2	100.4	nd	nd	35869 ± 80	nd

HC = healthy control, LE = left eye, nd = not detected, RE = right eye

infection.[9,10] A large-scale study demonstrated elevated levels of proinflammatory cytokines such as IFN-γ, tumor necrosis factor (TNF), IL-5, IL-8, and GM-CSF in the tears of patients with confirmed SARS-CoV-2 detection in the conjunctival sac.[11]

Our report details a patient with no preexisting eye issues, who had mild, nonspecific ocular surface symptoms 10 days after a COVID-19 infection, experiencing an acute change in her vision, which developed myopic astigmatism in both eyes. In addition, her husband developed a rare neurologic disease, acute disseminated encephalomyelitis, which led to his death. Despite the patient's refusal to undergo conjunctival and corneal tissue biopsy, the response to topographic, OCT, and elevation changes of ocular surface inflammatory markers was inferred from the corresponding expression of ACE2 and TMPRSS2 host receptors on the ocular surface for COVID-19.

These acute changes that were secondary to COVID-19 affected the ocular surface by elevating inflammatory and immunological mediators, which were detectable only through molecular biology techniques in specialized laboratories and resulted in alterations in the corneal epithelium and superficial stroma.

The abnormal thickness of the corneal epithelium may be a pathologic response to direct virus infection through ACE receptors. To evaluate the activity of the ocular surface in seemingly unaffected eyes during slit-lamp examination, we conducted cytokine, chemokine, and MMP tests on tear samples from the patient and controls. In contrast to the tear fluid from HCs, the patient's tears from both eyes demonstrated detectable levels of IFN-β, a cytokine involved in antiviral immunity, as well as elevated levels of IL-6, IL-1β, and chemokines IP-10 and IL-8, which were correlated with increased pro-MMP-9 activity. MMP-9 belongs to the family of matrix MMPs, zinc endopeptidases primarily involved in extracellular remodeling. In the eye, MMPs are normally expressed at low levels under normal conditions.[12,13]

However, MMP-9 has been found to be overexpressed in models of dry eye disease, corneal ulceration, microbial keratitis, and other surface pathologies. Its overexpression is associated with increased apoptosis, oxidative stress, and a disruption of the epithelial barrier integrity. Inhibiting MMP-9 using physiologic inhibitors or anti-inflammatory drugs often attenuates these biological events, leading to a reduction in ocular surface disease.[14]

Interestingly, tissue inhibitors of MMPs (TIMPs), which prevent the conversion of pro-MMP-9 to active MMP-9 and are frequently found in MMP-9/TIMP complexes, are also upregulated in inflamed corneas, albeit with delayed kinetics. In our study, we observed a high-molecular-weight band of 105 kDa, likely representing MMP-9/TIMP complexes, in addition to the 92-kDa pro-MMP-9 band, in all samples, particularly in the tears of the patient's left eye (data not shown). It is worth noting that the function of MMP-9 may vary depending on the context, as it has been shown to exert defensive anti-inflammatory responses in certain conditions.[15]

Both IL-1β and IL-6 have been shown to stimulate the production of MMPs.[16] The local proinflammatory environment observed in our study resembles the concept of a "cytokine storm" and pathogenic inflammation associated with severe clinical manifestations of COVID-19.[17] Therefore, corneal SARS-CoV-2 infection in this patient may have led to persistent type I IFN, along with excessive production of proinflammatory cytokines and chemokines, which sustained immune cell recruitment and tissue damage, contributing to the pathologic changes in the corneal epithelium.

Cytokines play a crucial role in maintaining ocular surface health and homeostasis by regulating inflammation, immune responses, and tissue repair. In the tear film, cytokines such as interleukins (IL-1, IL-6), TNF-α, and IFNs (IFN-γ) are involved in modulating immune cell activity, promoting epithelial cell proliferation, and regulating tear production. Dysregulation of cytokine levels can lead to conditions like dry eye disease, inflammation, and corneal damage. Understanding cytokine dynamics at the ocular surface and tear film is essential for managing ocular surface diseases and developing targeted therapies.

Several studies have evaluated the efficacy of topical CyA in modulating the transcript levels of inflammatory cytokines and extracellular matrix proteins in corneal epithelial cells and stroma. Cyclosporine is a medication commonly used to treat ocular surface diseases. It works by suppressing inflammation and modulating the immune response on the ocular surface, helping to improve tear film stability and corneal wound healing. In our study, we demonstrated that 2 months of topical CyA treatment "normalized" the concentration of inflammatory cytokines and pro-MMP-9. Although improvements in topography analysis were observed, the patient's preinfection refraction was not fully recovered, with a remaining myopia of −3.50.

In conclusion, we have presented a rare case report of a sudden shift to myopic astigmatism and topography alterations indicative of corneal epithelial irregularities (abnormal corneal steepening CLMI index, Galilei G6) in a patient who experienced mild COVID-19 symptoms. This was associated with upregulated cytokines and the presence of pro-MMP-9. It is known that corneal epithelial cells increase the secretion of cytokines and chemokines in response to cellular stimulation.

In the case described here, with an irregular and thickened corneal epithelium, along with significant keratometry and refractive changes following COVID-19 infection, it is logical to investigate alterations in inflammatory markers in the tear film and consider a treatment to normalize the ocular surface. Although the exact pathophysiologic mechanisms underlying viral invasion of the cornea, tissue structural alterations, and cytokine response are still poorly understood, ophthalmologists should maintain a high level of suspicion when symptoms suggestive of corneal involvement arise in patients recently infected with SARS-CoV-2.

It is noteworthy that this patient was monitored for an approximate period of 2 years, and her symptoms did not reappear until his last check-up, both at the biomicroscopic and topographic levels. The prevalence of ocular symptoms associated with COVID-19 has been a topic of ongoing research, with varying findings reported over time. Initially, studies suggested a relatively low prevalence of ocular symptoms, such as conjunctivitis, in COVID-19 patients. However, as more data accumulated and awareness increased, subsequent studies reported higher rates of ocular involvement.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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