

## Comparison of anterior segment parameters, corneal endothelial cell density, and coefficient of variation in COVID-19 and healthy groups

### Comparación de los parámetros del segmento anterior, la densidad de células endoteliales corneales y el coeficiente de variación en los grupos de COVID-19 y sanos



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#### Abstract

A total of 34 cases and 34 healthy individuals were included in this study. The patient group had a history of COVID-19 infection and hospitalization. They underwent full optometric examinations on their right eye, which included, autorefractometry, retinoscopy, ophthalmoscopy, slit lamp, and examinations with specular microscope and Pentacam devices. The average corneal endothelial cell density was  $2503.78 \pm 311.76$  in the patient group and  $2831.72 \pm 276.65$  in the healthy group, which indicated a significant decrease in the patient group, compared to the healthy group. There was a significant difference in the endothelial cell density between the two groups ( $P < 0.001$ ). Moreover, the coefficient of variation of cells was 36.63 in the healthy group and 37.00 in the patient group, which was raised. No significant differences were observed in the other anterior segment parameters, such as the central corneal thickness, anterior chamber angle, keratometry, corneal horizontal diameter, and pupil diameter ( $P > 0.05$ ). Individuals who have previously overcome COVID-19 experienced a reduction in the corneal endothelial cell density and an increase in cell coefficient of variation, as revealed by tests using a specular microscope and Pentacam devices. These findings could contribute to our comprehension of the systemic impacts of COVID-19 on the body.

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#### Resumen

Un total de 34 casos y 34 individuos sanos fueron incluidos en este estudio. El grupo de pacientes tenía antecedentes de infección por COVID-19 y hospitalización. Se sometieron a exámenes optométricos completos en su ojo derecho, que incluyeron, autorrefractometría, retinoscopia, oftalmoscopia, lámpara de hendidura y exámenes con microscopio especular y dispositivos Pentacam. La densidad celular endotelial corneal promedio fue de  $2503.78 \pm 311.76$  en el grupo de pacientes y de  $2831.72 \pm 276.65$  en el grupo sano, lo que indicó una disminución significativa en el grupo de pacientes, en comparación con el grupo sano. Hubo una diferencia significativa en la densidad celular endotelial entre los dos grupos ( $P < 0.001$ ). Además, el coeficiente de variación de las células fue de 36.63 en el grupo sano y de 37.00 en el grupo de pacientes, que aumentó. No se observaron diferencias significativas en otros parámetros del segmento anterior, como el grosor corneal central, el ángulo de la cámara anterior, la queratometría, el diámetro horizontal corneal y el diámetro pupilar ( $P > 0.05$ ). Las personas que habían superado la COVID-19 experimentaron una reducción de la densidad celular endotelial corneal y un aumento del coeficiente de variación celular, según se reveló mediante pruebas con microscopio especular y dispositivos Pentacam. Estos hallazgos podrían contribuir a nuestra comprensión de los efectos sistémicos de la COVID-19 en el organismo.

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## Introduction

COVID-19 was declared a global pandemic by the World Health Organization (WHO) on March 11, 2020, and was officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>1</sup>. While primarily affecting the respiratory system, SARS-CoV-2 has also been shown to impact multiple organs, including the ocular surface<sup>2</sup>. Recent concerns have emerged regarding the potential for ocular transmission of the virus, as viral particles have been detected in tear samples and conjunctival swabs of infected individuals<sup>3</sup>.

SARS-CoV-2 can persist and replicate in the conjunctiva even in the absence of eye disease, suggesting that asymptomatic individuals may contribute to virus transmission through ocular secretions<sup>4</sup>. Reports indicate that a significant proportion of COVID-19 patients develop conjunctivitis, positioning it as a possible early clinical sign of infection<sup>5</sup>. Additionally, the virus can be transmitted via respiratory droplets or airborne particles that come into contact with the corneal epithelium, further supporting concerns about ocular involvement in disease spread<sup>6</sup>.

Several studies have documented ocular manifestations of COVID-19, including dry eye symptoms resulting from tear film instability. This instability may be attributed to viral activity on the epithelial cells of the cornea and lacrimal glands<sup>7</sup>. Furthermore, SARS-CoV-2 may infiltrate the corneal nerve, reducing corneal sensitivity<sup>8</sup>. Similar to other respiratory viruses—such as adenovirus, influenza virus, herpes simplex, herpes zoster, and cytomegalovirus—SARS-CoV-2 may exhibit an affinity for ocular tissue, with potential effects on the corneal endothelium<sup>9,10</sup>. However, only a limited number of viruses are known to cause inflammation of corneal endothelial cells<sup>11</sup>.

The corneal endothelium is a monolayer of hexago-

nal, non-regenerating cells located on the posterior surface of the cornea. These cells actively regulate corneal hydration via sodium-potassium ATPase pumps, which prevent stromal swelling. Damage or loss of endothelial cells impairs this pump function, leading to corneal edema, loss of transparency, and reduced visual acuity<sup>12</sup>. Normal endothelial cell density (ECD) declines at an annual rate of 0.3-0.5 %, with healthy adults typically exhibiting (2000-2500 cells mm<sup>-2</sup>)<sup>13,14</sup>. Despite growing evidence of ocular involvement in COVID-19, the impact of SARS-CoV-2 on corneal endothelial cells remains underexplored.

This study aimed to assess the effect of SARS-CoV-2 on corneal endothelial cell density and anterior segment parameters. Given the limited research in this field, this study was crucial for understanding the long-term ocular implications of COVID-19.

## Materials and methods

*Study design.* This cross-sectional study was conducted in Mashhad, Iran, in 2023. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (ID: IR.MUMS.FH MPM.REC.1402.006). After obtaining approval from the Department of Optometry and the Research Council of the university, the research was carried out at the Toos Ophthalmology Clinic. Written informed consent was obtained from all participants.

*Sample size.* The minimum sample size was calculated based on a previous study by Oren *et al.*<sup>15</sup>, considering an error margin of 5 % and a statistical power of 80 %, using the average formula for two independent populations. The sample size was initially determined to be 5 participants per group based on the coefficient of variation (CV) of the cell area

and 15 participants per group based on the percentage of hexagonal (HEX) cells. Ultimately, 34 participants were included in each group.

**Inclusion and exclusion criteria.** The study established specific inclusion and exclusion criteria to ensure a well-defined participant pool. For inclusion, the COVID-19 group comprised individuals with a confirmed history of COVID-19 infection, validated by polymerase chain reaction (PCR) testing and hospitalization. The control group consisted of healthy individuals with no history of COVID-19 infection, confirmed by three negative PCR tests conducted within the last two years. Exclusion criteria were applied to both groups to minimize confounding factors, including pre-existing ocular diseases, a history of previous ocular surgery, and systemic diseases known to affect corneal endothelial cells. These criteria were designed to maintain the integrity and focus of the study.

**Evaluation parameters.** i) Corneal endothelial parameters. ECD, CV, and HEX. ii) Anterior segment parameters. Central corneal thickness, anterior chamber depth, anterior chamber angle, keratometry, corneal horizontal diameter, and pupil diameter.

**Study procedure.** The study evaluated 34 right eyes of patients over a 6-month. Each week, 3-4 participants who had previously been infected with severe COVID-19 and were hospitalized were assessed. The inclusion criteria for hospitalization included young individuals with oxygen levels below 94 %, presenting with shortness of breath, chest pain, dizziness,

impaired consciousness, prolonged fever, and having at least two months since a negative PCR test. Full optometric examinations, including autorefractometry, retinoscopy, ophthalmoscopy, and slit-lamp evaluations, were conducted.

Corneal endothelial parameters (density and CV) were measured using the Specular Microscope (Topcon SP-3000P), while anterior segment parameters (central corneal thickness, anterior chamber angle, keratometry, corneal horizontal diameter, and pupil diameter) were assessed using the Pentacam (Oculus, Germany). These evaluations were performed on the right eye of 34 healthy individuals with a mean age of  $36.76 \pm 6.39$  years, who had no history of COVID-19 infection, confirmed by three negative PCR tests in the past two years. Both the COVID-19 and healthy groups had a history of vaccination.

**Statistical analysis.** Data were analyzed using SPSS software. Normality was assessed using the Kolmogorov-Smirnov test. Independent t-tests and Mann-Whitney U tests were employed for comparing groups, with a significance level set at  $p < 0.05$ . Descriptive and analytical methods were used to summarize the data, and results were presented through graphs, tables, and indices. The Chi-square test was applied to compare qualitative variables. The Shapiro-Wilk test was used to assess the normality of data distribution.

## Results

**Table 1. Evaluation of age, gender, and vaccination in the two groups**

		Patient (n=34)	Healthy (n=34)	P-value
<b>Age</b>		44.97±6.74	36.76±6.39	.000 <sup>a</sup>
<b>Gender</b>	Male	5	25	.000 <sup>b</sup>
	Female	29	9	
<b>Vaccination</b>	Yes	29	29	.321 <sup>c</sup>
	No	5	5	

This study was conducted with the participants of 68 participants, who were divided equally into two groups, including people who experienced and did not experience COVID-19. The mean age of participants in the COVID-19 group was higher than the group of healthy people. The first and third percentiles of age (years) in the study group were 39.5 and 50.25, and in the control, group were 31 and 40.5,

respectively. As Table 1 shows demographic information of study subjects, there were significant differences between the two groups of study regarding age and sex. However, regarding the injection of the COVID-19 vaccine, the two groups were completely similar.

Detailed results regarding the optometric examinations are presented in Tables 2 and 3 separately for two studied groups.

**Table 2. Optometric examinations in the group of patients who experienced COVID-19**

	N (Valid)	Mean	SD*	Percentiles		
				25	50	75
Pachyvertex	34	540.03	30.75	519.00	541.50	557.25
Keratometry	34	45.17	1.81	44.22	45.15	46.45
Corneal volume	34	59.46	3.61	56.33	60.20	61.73
The volume of the anterior chamber	34	135.94	30.53	117.00	128.00	161.75
Anterior chamber depth	34	3.20	.29	2.98	3.17	3.40
Eye pressure	34	.40	1.23	-.25	.35	1.20
HWTW**	34	11.58	.40	11.38	11.60	11.80
Anterior chamber angle	34	32.79	5.91	29.55	32.40	35.95
Pupil diameter	34	3.23	.61	2.83	3.05	3.56
Corneal thickness	34	.513	.033	.498	.508	.534
Number of cells	34	93.59	18.57	85.00	98.50	106.50
Average cell size	34	406.42	58.72	358.15	388.65	427.95
Standard deviation of cell size	34	147.89	27.49	126.35	146.75	158.68
Coefficient of variations in cell size	34	37.00	5.67	33.18	35.80	40.10
Density of cells	34	2503.78	311.76	2337.33	2573.15	2792.23
Hexagonality	34	50.76	8.85	44.00	51.00	56.00

\*SD: Standard deviation, \*\*HWTW: white-to-white corneal diameter

**Table 3 Optometric examinations in the group of patients who did not experience COVID-19**

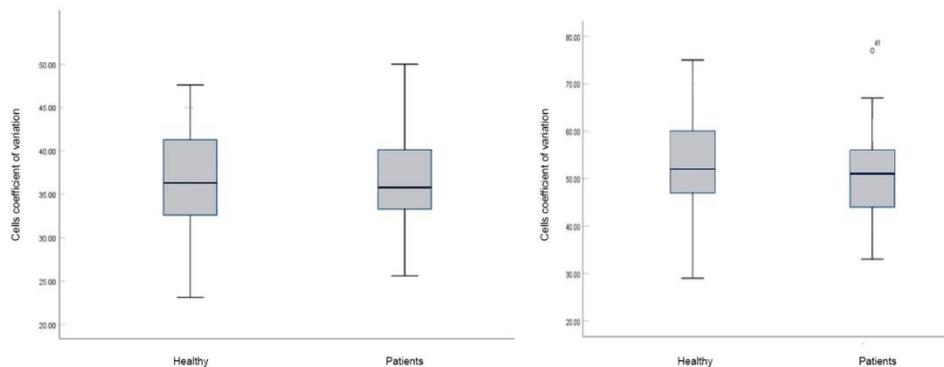
	N (Valid)	Mean	SD*	Percentiles		
				25	50	75
Pachyvertex	34	539.18	32.61	510.00	535.50	568.25
Keratometry	34	45.21	2.55	43.78	44.75	46.25
Corneal volume	34	60.24	3.37	57.65	59.85	62.53
The volume of the anterior chamber	34	163.91	34.76	137.50	160.00	180.00
Anterior chamber depth	34	3.49	.32	3.30	3.48	3.66
Eye pressure	34	.44	1.30	-.73	.60	1.60
HWTW**	34	11.76	.38	11.40	11.80	12.00
Anterior chamber angle	34	35.52	5.82	29.65	36.65	40.08
Pupil diameter	34	3.62	.70	3.01	3.68	4.11
Corneal thickness	34	.512	.032	.492	.508	.535
Number of cells	34	107.88	16.31	94.50	107.50	120.25
Average cell size	34	356.46	35.17	323.73	354.50	381.60
Standard deviation of cell size	34	131.77	22.32	113.75	136.70	148.20
Coefficient of variations in cell size	34	36.63	5.77	32.40	36.30	41.53
Density of cells	34	2831.72	276.65	2620.88	2820.80	3089.20
Hexagonality	34	52.85	10.26	46.75	52.00	60.25

\*SD: Standard deviation, \*\*HWTW: white-to-white corneal diameter

**Table 4 Comparison of anterior segment parameters and endothelial cell morphology between the two groups**

	Patient (n=34)	Healthy (n=34)	P-value
Cell density	2503.78±311.76	2831.72±276.65	.000 <sup>a</sup>
Coefficient of variation of cells	37.00±5.67	36.63±5.77	.789 <sup>a</sup>
Hexagonality	50.76±8.85	52.85±10.26	.372 <sup>a</sup>
Corneal thickness	.513±.03	.512±.03	.879 <sup>a</sup>
Anterior chamber angle	32.79±5.91	35.52±5.82	.059 <sup>a</sup>
Horizontal diameter of the cornea	11.58±.40	11.76±.38	.060 <sup>a</sup>
Maximum keratometry	45.17±1.81	45.21±2.55	.380 <sup>b</sup>
Pupil diameter	3.23±.61	3.62±.70	.230 <sup>b</sup>

a: Student t-test, b: Mann-Whitney U test

**Figure 1 Box plots of the difference in the morphology of corneal endothelial cells between the two groups by specular microscopy**

The results of anterior segment parameters and endothelial cell morphology were compared between the two groups (Table 4). The mean corneal ECD was obtained at 2503.78±311.76 in the patient group and 2831.72±276.65 in the healthy group, which indicated a significant decrease in the patient group, compared to the healthy group. There was a significant difference in the ECD between the two groups ( $P < 0.001$ ). The CV of cells was 36.63 in the healthy group and 37.00 in the patient group, which showed an increase. There was no significant difference in other anterior segment parameters, such as central corneal thickness, anterior chamber angle, keratometry, corneal horizontal diameter, and pupil diameter ( $P > 0.05$ ).

## Discussion

The findings of this study indicated individuals who have recovered from COVID-19 exhibit significant alterations in corneal endothelial cell morphology, including a reduction in ECD and hexagonal cell percentage (pleomorphism), as well as an increase in the CV of cell size (polymegathism), Oren & Kocabas<sup>16</sup> analysed 68 participants, including 34 healthy controls and 34 individuals who had recovered from COVID-19. They reported a significant reduction in corneal ECD and an increase in CV within the infected group, accompanied by a decrease in hexagonal cells and an elevation in central corneal thickness, which may be attributed to impaired sodium-potas-

sium pump function<sup>15</sup>. Similarly, Erdem *et al.*<sup>17</sup>, conducted a study with 80 recovered COVID-19 patients and 72 healthy individuals, documenting a decrease in ECD, a rise in CV, a reduction in hexagonal cells, and an increment in central corneal thickness<sup>16</sup>. Kaushik *et al.*<sup>18</sup>, reported comparable findings in their study of 129 post-COVID patients and 123 healthy controls, further supporting the observed endothelial alterations<sup>17</sup>.

In contrast, in 2023 Elshalkami *et al.*<sup>19</sup>, examined 64 recovered individuals and 53 healthy controls and reported no statistically significant differences in endothelial parameters between the two groups. These discrepancies could be due to variations in patient grouping, differences in examination timing, or inconsistencies in the measurement techniques used in their study<sup>18</sup>.

The underlying mechanisms of these changes is still unclear; however, potential explanations might be direct viral invasion of corneal endothelial cells, systemic inflammatory responses, or hypoxic conditions during severe COVID-19 infections that may contribute to endothelial dysfunction. Additionally, previous studies have demonstrated that ocular manifestations of COVID-19, such as conjunctivitis and dry eye syndrome, could be linked to viral effects on tear film stability and corneal sensitivity<sup>7,19,20</sup>. The primary function of endothelial cells is maintaining the corneal transparency by regulating stromal hydration. Subsequently, the decline in ECD can lead to corneal decompensation, resulting an elevation in corneal thickness and subsequent potential visual impairment<sup>23-25</sup>.

The present study adds to the growing body of evidence indicating the impact of COVID-19 on corneal endothelial health. The observed reduction in ECD, increase in CV, and decrease in hexagonal cell percentage suggest that endothelial stress may persist

even after recovery from the infection. Assessing central corneal thickness can serve as an indirect measure of endothelial function, as previous studies have shown that reduced ECD and impaired sodium-potassium pump activity may lead to increased corneal thickness due to disrupted fluid regulation<sup>26,27</sup>. These findings contribute meaningfully to the expanding body of evidence regarding the systemic nature of COVID-19, reaffirming that its effects extend beyond the respiratory system. Increasingly, research has documented the virus's multi-organ involvement, including its ocular manifestations. The corneal endothelium, a critical and non-regenerative layer essential for maintaining corneal clarity, appears to be susceptible to post-infectious alterations. In terms of physiopathology of the condition, in post-mortem examinations of corneal donors, SARS-CoV-2 RNA as well as envelope and spike proteins were detected in the corneal tissues of both COVID-19 positive and asymptomatic individuals, with detection rates of 11% and 15%, respectively. Notably, the highest prevalence of viral RNA was observed on the posterior (endothelial) surface of the cornea up to 25 %<sup>29</sup>.

In light of the potential for future pandemics caused by similar viral pathogens, a deeper understanding of the long-term impact of COVID-19 on corneal endothelial health is imperative. Our results underscore the importance of ongoing ophthalmologic monitoring in recovered individuals. Future research should prioritize longitudinal investigations to determine the persistence of these endothelial changes and assess whether they may predispose patients to subsequent corneal pathologies. Enhanced insight into these mechanisms may ultimately guide more effective post-COVID ocular care strategies.

In summary, although transient alterations in corneal endothelial parameters have been reported in the

early post-infectious period, the study by Elshalkami et al.<sup>19</sup>, suggests that these parameters may normalize over time. Specifically, their findings indicate that corneal endothelial cell characteristics in individuals recovered from SARS-CoV-2 infection were comparable to those of healthy controls six months or more after recovery, implying a lack of sustained or permanent endothelial damage. Nevertheless, further large-scale longitudinal studies are warranted to confirm these findings and to exclude subtle or delayed effects<sup>19</sup>.

This study supports the hypothesis that COVID-19 can induce long-term alterations in corneal endothelial morphology. However, the relatively small sample size and short follow-up duration represent key limitations. Further studies with larger cohorts and extended follow-up periods are necessary to determine the clinical relevance of these findings.

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### **Conflicts of interest**

The authors declare no competing interests.

### **Acknowledgments**

Not applicable.

### **Availability of data and materials**

The datasets analyzed in this study are available upon reasonable request from the corresponding author

### **Ethical considerations**

The research adhered to the tenets of the Declaration of Helsinki and Written informed consent was obtained from the participants in the present study. Also, the Ethics Committee of Mashhad University of Medical Sciences approved the study with ID IR.MUMS.FHMPM.REC.1402.006.

### **Informed Consent**

Written informed consent was obtained from all participants.

### **Research limitations**

The study has several limitations, including a small sample size, which may restrict the generalizability of the findings and reduce the statistical power to detect significant effects. Additionally, there is a lack of longitudinal follow-up, which hinders the ability to assess long-term endothelial changes and understand the sustained impact of the intervention over time. These limitations highlight the need for further research with larger cohorts and extended observation periods to draw more robust conclusions.

### **Authors' contributions**

*Heravian Shandiz Javad, Ostadi Moghaddam Hadi*, study design. Etemadi Majd Amir, Saffari Rahim, data collection. Jamali Jamshid, data analysis. Azimi Khorasani Abbas, Etemadi Majd Amir, manuscript preparation.

### **Consent for publication**

Not applicable.

## Use of artificial intelligence

We assume that the entire document was written based on ethical and professional criteria, and AI was not used to make the images or text.

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