REVIEW

Assessment of corneal endothelial cell parameters using specular microscopy in previously infected SARS-CoV-2 patients

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Abstract

Purpose   To examine the long-term effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on the corneal endothelium.

Methods   This was a comparative, cross-sectional study that included subjects who had recovered from SARS-CoV-2 infection for at least 6 months (group 1) and a group of age- and sex-matched controls with no prior symptomatology or documentation of SARS-CoV-2 infection (group 2). After full ophthalmological evaluation, specular microscopy was used to examine the endothelial cell parameters, including endothelial cell density, coefficient of variation, hexagonality, average area, and central corneal thickness.

Results   Sixty-four and 53 right eyes were included in groups 1 and 2, respectively. No statistically significant differences were detected in any of the examined specular parameters between the two groups.

Conclusion   SARS-CoV-2 infection may have no delayed sequel on the corneal endothelium. Future prospective studies with repeated examinations in the same subjects would be useful.

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Introduction

The endothelial cell layer consists of a single layer of hexagonal cells on the back surface of the cornea that face the anterior chamber and plays a crucial physiological role in regulating corneal stromal hydration and, consequently, its transparency [1]. An adequate number of endothelial cells are required to maintain clear vision; the number of cells varies according to age since the cells are incapable of mitotic replacement, and is thus highest at birth ($\approx$ 3500 cells/mm^2), and averages at 1500–3000 cells/mm in adults [2, 3]. Multiple other factors affect endothelial cell count, such as trauma, ocular and systemic diseases and ocular surgery [4–6]. Compensatory mechanisms in response to the loss of endothelial cells include enlargement of healthy cells (polymegathism) to replace the defects on the corneal back surface, with a consequent reduction in their hexagonality (pleomorphism) [7]. Below a certain critical number that varies according to each individual (but is generally less than 500 cells/mm^2), corneal endothelial decompensation would result in excessive hydration of the cornea, known as corneal edema, with increased stromal thickness, bullae formation and eventual scarring,

all leading to reduced visual acuity and eye discomfort that could be irreversible [8, 9].

Specular microscopy provides a non-invasive, non-contact means to assess corneal endothelial characteristics. The analysis includes measurement of the endothelial cell density (ECD) which is the average number of cells counted by the instrument per squared millimeter, hexagonality (HEX) which measures the degree of morphological uniformity of the cells reflecting on the degree of pleomorphism, and coefficient of variation (CV) which reflects the degree of variation in cell size (polymegathism) [10]. Another parameter that could be obtained by specular microscopy imaging is the central corneal thickness (CCT) which is the true indicator of whether corneal decompensation has initiated [11]. Less indicative parameters in the machine's output include the standard deviation (SD) from the average cellular area (AVG) and the maximum (MAX) and minimum (MIN) number of cells that can be automatically counted in a single field [10].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for the recent coronavirus disease 2019 (COVID-19) pandemic. The encapsulated, single-stranded, RNA virus processes a spike glycoprotein, which the virus utilizes to enter host cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor [12]. The ACE2 receptor has been demonstrated to be expressed in many tissues of the body, including corneal and conjunctival tissue which has supported the hypothesis for the ocular route of viral transmission [13]. Moreover, CD147 (also known as Basigin or extracellular matrix metalloproteinase inducer) expression has been associated with promotion of SARS-CoV-2 cellular invasion, and the glycoprotein has also been shown to be expressed in human ocular tissue including the cornea, tears, aqueous humor and vitreous fluids [13]. A recent study [14] has claimed a decrease in corneal ECD and increased HEX in patients that had recovered from COVID-19 in comparison with healthy controls. The findings of the study have not been corroborated elsewhere.

The aim of our work was to assess whether there was a significant correlation between endothelial cell parameters and previous SARS-CoV-2 infection.

Methods

This comparative, cross-sectional study was conducted in Ain Shams University Hospitals over the period of 6 months, from January 2021 till July 2021. The study was conducted according to the tenets of declaration of Helsinki and received approval of the ethical and scientific committees of Ain Shams University. All included subjects signed a comprehensive written consent prior to participation in the study.

The presented study included 64 right eyes of subjects previously infected by SARS-CoV-2 (group 1) and 53 right eyes of control subjects who gave no history of previous infection by SARS-CoV-2 (group 2). Group 1 had subjects who recovered from COVID-19 six or more months earlier as documented by a prior positive result of reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2. They were matched to the control group with similar age range (18 to 60 years) and sex distribution. Subjects were excluded from the study if their medical or ocular history/examination included any factor or condition reported to affect corneal endothelial parameters, including diabetic mellitus, hypertension, chronic kidney or liver disease, heart failure, pregnancy, smoking, corneal opacity, contact lens wearing, high error of refraction (spherical equivalent more than $+6$ or -6 diopters), previous intraocular surgery or trauma, glaucoma or uveitis.

All subjects underwent full ophthalmological evaluation including corrected distance visual acuity (CDVA), intraocular pressure (IOP) measurement, slit lamp biomicroscopy, and dilated fundus examination. The subjects' corneal endothelial parameters were then evaluated using the CEM-530 specular microscope (NIDEK Co., Ltd, Japan). The following parameters were recorded from the central corneal area: ECD, AVG, SD, CV, HEX, CCT, MAX and MIN.

The sample size was calculated to obtain enough statistical power for the study, and it revealed that group sample sizes of at least 45 and 45 achieve 80% power to reject the null hypothesis of zero effect size when the population effect size is 0.60 and the significance level (alpha) is 0.050 using a two-sided two-sample equal-variance t-test. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version number 23, Microsoft Excel and GraphPad Prism 8 program. Statistics included

means, standard deviations (SD) and unpaired *t*-test (Welch's correction was applied when needed). A *p*-value < 0.05 was considered statistically significant.

Results

The mean age of the subjects in group 1 was 41.11 ± 8.97 years and in group 2 was 42.02 ± 11.16 years (*p* = 0.6332). Half of the subjects in group 1 and 45.3% (*n* = 24) of the subjects in group 2 were males.

All subjects in both groups had a best corrected visual acuity (BCVA) of 0.8 (Snellen equivalent 20/25) or better. The mean IOP in group 1 was 15.4 ± 2.6 mmHg and in group 2 was 15.6 ± 2.7 mmHg (*p* = 0.6419). The mean spherical equivalent among group 1 subjects was −0.14 ± 0.78 diopters and among group 2 subjects was −0.02 ± 0.87 diopters (*p* = 0.4334).

Corneal endothelial cell parameters did not show any significant difference between post-COVID cases and controls as given in Table 1.

Table 1 demonstrates the distribution of the corneal endothelial cell parameters among both groups. No statistically significant differences were found regarding any of the measured variables between both groups.

Discussion

We analyzed the endothelial cell parameters using specular microscopy in a group of individuals with prior COVID-19 and compared them to a group of age- and sex-matched controls. We did not find any significant difference between both groups as regards the endothelial cell variables, opposing the theory of permanent corneal endothelial affection by the viral infection.

Endothelial cell involvement is a known feature of other ocular viral infections that have been shown to induce endothelial inflammation (endotheliitis), whether transient or permanent, that leads to variation of endothelial parameters on imaging. Both the herpes simplex virus [15] and cytomegalovirus [16] have been demonstrated to cause a reduction in ECD. Systemic viral infections have also been evidenced to affect the corneal endothelium, such as the human immunodeficiency virus, which has been shown to contribute to corneal endothelial polymegathism [17]. The latter finding was attributed to the generalized early cellular senescence caused by the virus [17]. If the SARS-CoV-2 is hypothesized to affect the corneal endothelium, the direct ocular inflammatory route would be more likely since the endothelial cells possess the necessary surface receptors that would allow viral invasion [12], a mechanism that has been at the center of the ocular route theory of viral transmission [13].

To date, only one study [14] has examined the effect of SARS-CoV-2 infection on the corneal endothelium. In their work, Erdem and colleagues also utilized specular microscopy to examine the corneal endothelial parameters in a group of individuals who had recovered from the viral infection for at least one month and compared them to a control group who had no prior documentation of the viral illness. They found a statistically significant difference in all endothelial cell parameters between both groups,

Table 1 Comparison of central endothelial cell parameters between both groups

CV coefficient of variation, *ECD* endothelial cell density, *HEX* hexagonality index, *AVG* average area, *MIN* minimum area, *MAX* maximum area, *SD* standard deviation of area, *CCT* central corneal thickness

*Statistically significant

Variable	Group 1 (cases) *n* = 64 Mean ± SD	Group 2 (control) *n* = 53 Mean ± SD	*P* value (unpaired t test)
CV	29.66 ± 3.88	30.59 ± 4.49	0.2387
ECD (cells/mm^2)	2943 ± 197.89	2925 ± 270.63	0.7029
HEX (%)	59.34 ± 7.54	59.04 ± 8.88	0.8430
AVG (um^2)	341.4 ± 24.3	344.7 ± 33.3	0.5460
MIN (um^2)	147.8 ± 13.22	147.91 ± 19.31	0.9723
MAX (um^2)	729.34 ± 120.61	731.45 ± 115.41	0.9234
SD (um^2)	101.47 ± 16.98	106.11 ± 24.25	0.2427
CCT (um)	520.77 ± 21.01	516.98 ± 27.18	0.4091

with lower ECD and HEX, and higher CV, AVG and CCT in the previously infected group. The proposed explanation by the authors was the pro-inflammatory properties of SARS-CoV-2 which may have caused a form of viral endotheliitis with transient impairment of the endothelial sodium–potassium pump, which is responsible for maintaining corneal transparency and protection against corneal edema (the impairment being reflected as increased CCT) [14].

Although our study design, period and sample size are comparable to the previous publication by Erdem and colleagues [14], our results are in stark contrast to theirs. Our study group, however, included subjects who had recovered from COVID-19 for at least 6 months, while Erdem et al. [14] included those who had recovered for at least 1 month, with a mean (SD) duration from a negative RT-PCR of $38.45(\pm 6.87)$ days. The effect on the corneal endothelium could, thus, be transient and early, related to an inflammatory process by the viral infection as proposed by the authors [14], with late normalization of the endothelial surface. This would agree with Hillenaar et al [15], who found an improvement in endothelial changes after herpes simplex viral endotheliitis as pseudoguttata, enlarged intercellular gaps, loss of defined cell boundaries, spot-like holes and endothelial denudation after the 100-day follow-up period although the EDC remained affected. Other explanations of the variability in our results may include the difference in studied population, with inherent variability in endothelial parameters reported in each specific population [18, 19], or difference in the viral strain among the different geographical locations [20] and/or the immune response of the different groups to the infection [21]. Another case report hypothesizes that the cause of its patient's corneal decompensation was viral endotheliitis due to direct infection by the SARS-CoV-2 virus [22].

It is to be noted that both the study of Erdem et al. [14] and our study had a cross-sectional design, with the subject allocation to either group being based on history taking and examining prior documentation of the viral infection. Most of SARS-CoV-2 infections are asymptomatic or cause mild symptoms that may pass unnoticed or may be mistaken for another mild illness [23]. This makes it difficult to validate the lack of prior infection in the control group and may have caused an overlap between subjects in the two groups, a major limitation to both studies. An optimum design would be one that allows repeated examination of the corneal endothelial cell parameters in the same subjects prior to the infection, during and shortly after the infection, and late after recovery, to detect any transient and permanent changes that could be related to the viral infection.

In summary, our results suggest that corneal endothelial cell parameters in subjects previously infected with the SARS-CoV-2 could be comparable to those of healthy controls 6 or more months after recovery from the infection, suggesting a lack of a delayed, permanent effect of the virus on the corneal endothelium.

Declarations

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