Corneal endothelium, retinal nerve fiber layer, ganglion cell complex, and perimetry measurements in normal eyes and those with primary open-angle glaucoma

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ABSTRACT

Background: Corneal endothelial cell (CEC) loss in glaucoma can be attributed to the direct compressive effect of elevated intraocular pressure. Herein, we aimed to evaluate specular microscopic changes in CEC count and morphology in correlation to retinal nerve fiber layer (RNFL) changes detected by spectral-domain optical coherence tomography (SD-OCT) in early and advanced primary open-angle glaucoma (POAG).

Methods: This descriptive-analytical study involved patients with medically controlled POAG versus non-glaucomatous patients of the same age group. Specular microscopy, visual field testing, and SD-OCT of the RNFL and macular ganglion cell complex (GCC) were performed. Eyes with POAG were further subcategorized into early and advanced stages.

Results: The study included 130 eyes of 130 participants; 70 were eyes with POAG (40 eyes with early-stage POAG, 30 eyes with advanced-stage POAG), and 60 were healthy eyes. The groups were comparable regarding mean age and sex. No significant difference was found in corneal parameters between healthy eyes, eyes with early POAG, and eyes with advanced POAG (all $P > 0.05$). In eyes with early-stage POAG, a significant negative correlation was found between the coefficient of variation (CV) and superior RNFL thickness ($r = -0.5$; $P = 0.018$), and between the percentage of hexagonal cells (hexagonality) and vertical cup-to-disc ratio ($r = -0.43$; $P = 0.035$). A significant positive correlation was found between hexagonality and superior as well as inferior RNFL thickness ($r = +0.53$; $P = 0.008$ and $r = +0.50$; $P = 0.015$, respectively). However, in the advanced glaucomatous eyes, no significant correlation was found between RNFL thickness and CEC parameters.

Conclusions: CEC parameters were not affected in eyes with early or advanced POAG compared with healthy eyes, despite a significant thinning of RNFL and macular GCC. In eyes with early-stage POAG, a significant correlation was found between morphological characteristics of CECs, such as CV and hexagonality, with superior and inferior RNFL thickness in the optic nerve head on SD-OCT images. Future longitudinal studies with larger sample sizes are needed to verify our results.

KEYWORDS

primary open angle glaucoma, chronic primary open angle glaucoma, corneal endothelium, retinal ganglion cell, perimetry, optic nerve head

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INTRODUCTION

The unique clarity and transparency of the cornea are dependent on its stromal anatomical structure and the function of the most inner hexagonal endothelial cell layer [1-3]. Corneal endothelial cell (CEC) loss is irreversible. Therefore, to maintain corneal transparency, preservation of these cells is mandatory [4]. Various conditions such as trauma, cataract surgery, diabetes mellitus, and glaucoma can insult the CECs, resulting in corneal decompensation and opacification [4-6].

Glaucoma is a potentially blinding disease that varies between primary and secondary types, and is associated with optic neuropathy and retinopathy in addition to its effect on CECs [7]. Endothelial cell loss in glaucoma can be attributed to the direct compressive effect of elevated intraocular pressure (IOP), the toxicity of medications, and the effect of surgeries and laser procedures used to control IOP [6, 8, 9]. Glaucoma and cataracts are more prevalent and commonly coexist among the aged. In this population, evaluation of CECs is mandatory to assess their ability to withstand the trauma of cataract surgery, of which CEC loss is an inevitable consequence [10, 11]. The effect of acute IOP increases on corneal endothelium was previously investigated [12-14]; however, little information is available about the effect of chronic IOP elevation on CEC count and morphology in eyes with primary open-angle glaucoma (POAG) [15].

Herein, we aimed to study the CEC count and morphology in eyes with early or advanced POAG compared with the corresponding variables in healthy eyes. Additionally, we determined any correlation between retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) thickness and CEC count and morphology in eyes with early or advanced POAG.

METHODS

In this descriptive-analytical study, the right eyes of patients in the same age group with medically controlled POAG or without POAG were recruited. The study received ethical approval from the Human Ethical Committee of the Sohag Faculty of Medicine (IRB registration number: Sohag-Med- 22-01-37). Written informed consent was obtained from all participants after an explanation of the study procedure. The tenets of the Helsinki Declaration were followed. All participants were chosen using a convenience sampling method from the attendees who fulfilled the inclusion criteria.

In the POAG group, IOP was controlled for at least 6 months with one or more of the following antiglaucoma medications: latanoprost 0.005% (Xalatan®; Pfizer, NY, USA), betaxolol 0.5% solution (Betoptic Solution, 0.5%®, Alcon, USA), Alphanova 0.15% ophthalmic solution (Brimonidine tartrate 0.15%, Orchidia, Cairo, Egypt), or brinzolamide 1% (Azopt; Alcon Laboratories, Fort Worth, TX). The study participants attended the Sohag Ophthalmic Investigation Center from June 2019 to December 2019 for their routine scheduled follow-up, which included visual field (VF) testing and spectral-domain optical coherence tomography (SD-OCT) for RNFL and macular GCC thickness measurement.

The Glaucoma Staging System 2 (GSS2) was used for the classification of glaucoma. It is based on two main perimetric global indices, mean deviation (MD) and pattern standard deviation (PSD), plotted on an X-Y coordinate. It classifies VF defects into six stages: stage 0 (normal population), 0 to 1 (borderline), and five consecutive stages from 1 to 5 according to damage severity. Classification also includes three types of defects (generalized, localized, and mixed) [16]

In this study, we considered stages 1 and 2 of the GSS2 as an early-stage subgroup of POAG and stages 3 to 5 as an advanced-stage subgroup. The mean (standard deviation [SD]) from the initial diagnosis to recruitment time in the current study was 3.5 (1.61) years and 4 (1.87) years for the early and advanced POAG subgroups, respectively. Participants with healthy eyes were examined twice for IOP measurement on two separate occasions. They were also tested for RNFL thickness and VF to exclude normal-tension glaucoma.

In the POAG group, we included eyes with the following: IOP medically controlled for at least six months; mean (SD) IOP of 15.5 (2.6) by Goldman applanation tonometry [17]; cup-to-disc ratio (C/D ratio) ≥ 0.7; cup asymmetry > 0.2 or difference in vertical C/D ratio and horizontal C/D ratio > 0.2; notching, disc hemorrhage, or excavation reaching the disc margin; glaucomatous VF changes (stages 1 to 5 according to GSS2); peripapillary RNFL thinning; and best-corrected distance visual acuity (BCDVA) of ≥ 0.4 logarithm of the minimum angle of resolution (logMAR) (Table 1).

We excluded eyes with juvenile open-angle glaucoma, angle-closure glaucoma, normal-tension glaucoma, pseudoexfoliation syndrome, chronic contact lens use, ametropia > ± 6 D, myopic fundus changes, media opacities, any neurological disorders, previous operations (including IOP-lowering surgeries and laser procedures), retinal pathologies, unreliable VF tests, and SD-OCT images of signal strength < 60.

Table 1. Baseline characteristics of the study participants

Variables	Healthy eyes (n = 60)	Early-stage POAG (n = 40)	Advanced-stage POAG (n = 30)	P-value
Age (y), Mean ± SD	59.5 ± 8.4	58.5 ± 9.42		0.500
Sex (Male / Female), n (%)	16 (26.66) / 44 (73.33)	21 (30) / 49 (70)		0.600
BCDVA (logMAR), Mean ± SD	0.77 ± 0.16	0.9 ± 1.37	0.47 ± 0.07	0.322
IOP (mmHg), Mean ± SD	13.05 ± 1.24	15.03 ± 3.11	14.59 ± 1.02	**0.010**

Abbreviations: POAG, primary open-angle glaucoma; n, number; y, years; SD, standard deviation; %, percentage; BCDVA, best-corrected distance visual acuity; logMAR, logarithm of the minimum angle of resolution; IOP, intraocular pressure; mmHg, millimeter of mercury. P-values < 0.05 are shown in bold. P-values were calculated using analysis of variance. Note: Early-stage POAG, stages 1 and 2 based on the Glaucoma Staging System 2; Advanced-stage POAG, stages 3 to 5 based on the Glaucoma Staging System 2.

Table 2. Corneal parameters in study participants as assessed by specular microscopy

Variables	Healthy eyes (n = 60)	Early-stage POAG (n = 40)	Advanced-stage POAG (n = 30)	P-value
CCT (μm), Mean ± SD	518.53 ± 44.14	538.4 ± 40.6	515.33 ± 16.9	0.174
ECD (cells/mm²), Mean ± SD	3085 ± 172.56	2943.9 ± 449.8	2958.73 ± 371.2	0.569
CV (%), Mean ± SD	40.5 ± 20.8	35.52 ± 4.30	38.20 ±7.54	0.454
Hexagonality (%), Mean ± SD	43.1 ± 14.6	45.04 ± 9.15	40.90 ± 7.93	0.622
Minimal cell size (μm²), Mean ± SD	118.16 ± 9.62	117.04 ± 9.81	110.5 ± 9.03	**0.030**
Maximal cell size (μm²), Mean ± SD	700.72 ± 132.5	810.09 ± 188.4	1211.7 ± 1155.7	0.060
Average cell size (μm²), Mean ± SD	337 ± 43.02	346.27 ± 70.41	350.5 ± 44.11	0.800

Abbreviations: POAG, primary open-angle glaucoma; n, number; CCT, central corneal thickness; μm, micrometer; SD, standard deviation; ECD, endothelial cell density; cells/mm², number of cells per square millimeter; CV, coefficient of variation; Hexagonality, the percentage of hexagonal cells; μm², square micrometers. P-values < 0.05 are shown in bold. P-values were calculated using analysis of variance. Note: Early-stage POAG, stages 1 and 2 based on the Glaucoma Staging System 2; Advanced-stage POAG, stages 3 to 5 based on the Glaucoma Staging System 2.

Table 3. Optical coherence tomography, optic disc, and visual field parameters of study participants

Variables	Healthy eyes (n = 60) Mean ± SD	Early-stage POAG (n = 40) Mean ± SD	Advanced-stage POAG (n = 30) Mean ± SD	P-value
Superior RNFL thickness (μm)	119.06 ± 26.64	110.064 ± 31.22	61.09 ± 24.014	**0.003**
Inferior RNFL thickness (μm)	124.064 ± 20.19	112.52 ± 36.42	60.36 ± 38.47	**0.003**
Temporal RNFL thickness (μm)	75.22 ± 12.13	66.98 ± 18.42	70.09 ± 36.04	**0.042**
Nasal RNFL thickness (μm)	76.42 ± 10.46	74.34 ± 21.037	71.32 ± 44.27	**0.033**
Average macular GCC thickness (μm)	96.64 ± 10.47	92 ± 13.34	67.54 ± 24.73	**0.001**
Superior macular GCC thickness (μm)	95.42 ± 9.33	90.58 ± 13.64	70 ± 22.54	**0.001**
Inferior macular GCC thickness (μm)	97.74 ± 13.14	93.29 ± 13.60	65.18 ± 26.97	**0.001**
MD of VF (dB)	- 1.63 ± 3.14	- 4.21 ± 1.28	- 19.899.86 ±	**0.001**
PSD of VF (dB)	2.10 ±2.33	3.68 ±1.43	8.38 ±3.20	**0.001**
Vertical C/D ratio	0.46 ±0.26	0.56 ±0.65	0.69 ±0.31	**0.001**
Cup volume (mm³)	0.20 ±0.28	0.34 ±0.232	0.27 ± 0.267	0.149

Abbreviations: POAG, primary open-angle glaucoma; n, number; SD, standard deviation; RNFL, retinal nerve fiber layer; μm, micrometer; GCC, ganglion cell complex; MD, mean deviation; VF, visual field; dB, decibels; PSD, pattern standard deviation; C/D ratio, cup-to-disc ratio; mm³, cubic millimeters. P-values < 0.05 are shown in bold. P-values were calculated using analysis of variance. Note: Early-stage POAG, stages 1 and 2 based on the Glaucoma Staging System 2; Advanced-stage POAG, stages 3 to 5 based on the Glaucoma Staging System 2.

All eyes were subjected to VF examination using the standard automated perimetry (SAP) Humphrey Field Analyzer (HFA) II 750 (Carl Zeiss Meditec Inc., Dublin, CA, USA) and the 30-2 test with Standard Swedish Interactive Thresholding Algorithm Strategy. SAP tests were classified according to the Anderson and Patella criteria [18].

SD-OCT with RTVue (Optovue Inc., Fremont, CA, USA) of the optic disc (optic nerve head [ONH]/ RNFL scan) and macular GCC (GCC segmentation) was performed.

Non-contact specular microscopic images (SP-2000P; Topcon Corporation, Tokyo, Japan) were captured from the central cornea (central measurement) and analyzed using IMAGENet 2000 software (Topcon). Endothelial cell density, percentage of hexagonal cells (hexagonality), coefficient of variation (CV), minimal cell size, maximal cell size, and average cell size were determined automatically. All examinations were performed by the same examiner who was blinded to the participants' clinical diagnoses. Multiple images were taken from a central view for each eye to obtain clear, high-quality images of the central endothelium, in which the centers of at least 100 contiguous endothelial cells with clear boundaries were marked using software available in the system.

IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity variances before further statistical analysis. Differences between groups for continuous measures were evaluated using analysis of variance, the independent sample t-test, and the chi-squared test for categorical measures. A post hoc Tukey test was performed for pairwise analysis whenever indicated. Pearson's correlation coefficients (r) were used to assess the correlation between the variables of corneal parameters, VF, and SD-OCT disc parameters. $P \leq 0.05$ was considered statistically significant.

RESULTS

The study included 130 eyes of 130 participants (70 eyes with POAG and 60 healthy eyes). The mean (SD) ages were comparable: 58.5 (9.42) in the POAG group and 59.5 (8.4) years in the healthy eye group ($P = 0.500$). Sex distributions were also comparable (30% men in the POAG group and 26.66% in the healthy eye group, $P = 0.600$). Table 1 shows the means (SDs) of age, BCDVA, and IOP, as well as sex distribution in participants with healthy eyes (60 eyes), early POAG (40 eyes), and advanced POAG (30 eyes), which did not differ significantly (all $P > 0.05$).

Table 2 presents the different parameters of specular microscopy, and no significant difference was found in corneal parameters between healthy eyes, eyes with early POAG, and eyes with advanced POAG (all $P > 0.05$).

Table 3 presents the SD-OCT optic disc and VF parameters in the study groups. A significant difference was found in all SD-OCT parameters, VF indices, and vertical C/D ratio between healthy eyes, eyes with early-stage POAG, and eyes with advanced-stage POAG (all $P < 0.05$), except for cup volume. Therefore, a post hoc test was performed for pairwise analyses.

The superior RNFL revealed a significant thinning in early and advanced glaucomatous eyes compared with that of healthy eyes (both $P < 0.001$), yet no significant difference was found between early and advanced glaucomatous eyes ($P > 0.05$). The inferior RNFL exhibited significant thinning in early and advanced glaucomatous eyes compared with that of healthy eyes (both $P < 0.001$), as well as in early glaucomatous eyes ($P = 0.05$) compared with that of advanced glaucomatous eyes. However, no significant difference was found in temporal or nasal RNFL thickness in pairwise analysis.

The superior macular GCC was significantly thinner in early and advanced glaucomatous eyes than in healthy eyes (all $P < 0.001$), yet no significant difference was found between early and advanced glaucomatous eyes (both $P > 0.05$). Inferior macular GCC thickness was not significantly different between early glaucomatous eyes and healthy eyes ($P > 0.05$). However, significant thinning was found in advanced glaucomatous eyes compared with healthy eyes ($P < 0.001$), as well as in advanced glaucomatous eyes compared with early glaucomatous eyes ($P < 0.001$).

Concerning VF indices, MD was significantly higher in early and advanced glaucomatous eyes than in healthy eyes (both $P < 0.001$), and in advanced glaucomatous eyes compared with early glaucomatous eyes ($P < 0.001$). PSD was significantly higher in early ($P = 0.004$) and advanced glaucomatous eyes ($P < 0.001$) than in healthy eyes, as well as in advanced glaucomatous eyes compared with early glaucomatous eyes ($P < 0.001$). The vertical C/D ratio was significantly greater in advanced glaucomatous eyes than in healthy eyes ($P = 0.003$).

In the early glaucomatous eyes, a significant negative correlation was found between CV and superior RNFL thickness ($r = -0.5$; $P = 0.018$) and between hexagonality and vertical C/D ratio ($r = -0.43$; $P = 0.035$). In addition, positive correlations were found between hexagonality and superior RNFL thickness ($r = +0.53$; $P = 0.008$) and inferior RNFL thickness ($r = +0.50$; $P = 0.015$). However, in eyes with advanced-stage POAG, no significant correlation was found between RNFL thickness and CEC parameters.

DISCUSSION

This study found no significant difference in CEC parameters, including CEC count, between healthy eyes, eyes with early POAG, and eyes with advanced POAG; yet, the mean count was lower in glaucomatous eyes. The superior and inferior RNFL, but not the temporal and nasal RNFL, were significantly thinner in early and advanced glaucomatous eyes than were those in healthy eyes. Superior macular GCC thicknesses was significantly less in early and advanced glaucomatous eyes than in healthy eyes. The inferior macular GCC was not significantly thinner in early glaucomatous eyes than that in healthy eyes; however, significant thinning was found in advanced glaucomatous eyes compared with healthy eyes. Significantly higher MD and PSD values, as well as vertical C/D ratio, were found in early and advanced glaucomatous eyes compared with healthy eyes. In the early glaucomatous eyes, significant correlations were found between CV and superior RNFL thickness, between hexagonality and vertical C/D ratio, and between hexagonality and superior and inferior RNFL thickness. These correlations were not found in the advanced glaucomatous eyes.

Previous studies have discussed corneal endothelial changes in relation to acute IOP elevation in angle-closure glaucoma (ACG), the use of antiglaucoma medications, and antiglaucoma surgical procedures [19-21]. We found no significant difference in corneal parameters between healthy eyes, eyes with early POAG, and eyes with advanced POAG with medically controlled IOP.

Our participants with healthy eyes and patients with POAG were comparable in terms of mean age, therefore age was excluded as a confounding factor, since POAG is usually seen at an older age and is associated with simultaneous age-related endothelial cell loss [22].

Our study revealed a significant negative correlation between CV and superior RNFL thickness and also between hexagonality and vertical C/D ratio. CV and hexagonality were affected in the early stages of glaucoma (morphological changes) and were associated with an increase in vertical C/D ratio with subsequent thinning of the superior RNFL that preceded the thinning of the inferior RNFL. These findings are consistent with those of Kim et al. [23], who concluded that thinning of the superotemporal RNFL is more correlated with and affected earlier by hypertension than the inferotemporal RNFL [23, 24]. The progressive increase in vertical C/D ratio was accompanied by progressive deterioration of CEC morphology (CV and hexagonality), while endothelial CEC density was not affected. CEC morphology changed in the form of an elevated or abnormal rate of polymegathism and a decreased number of functioning hexagonal endothelial cells as a result of the physiological stress to the corneal endothelium. These findings were different from those of Verma et al., who stated that morphological changes such as CV and hexagonality were not significantly different across the primary angle-closure spectrum [13]. This discrepancy in results may be due to the different natures of both types of glaucoma. ACG is characterized by acute attacks with severe and sudden closure of the angle and very high elevation of IOP, decreased visual acuity, and corneal edema, which could cause significant endothelial cell loss [25].

This study revealed an early effect on CV and hexagonality but not CEC density, which may be affected in the interval of chronic IOP elevation before glaucoma is diagnosed. Yu et al. reported that elevated IOP causes destruction of hexagonality, and the cell damage causes further deformation and loss; however, they did not correlate these CEC changes with RNFL or macular GCC thickness [15].

Morphological changes in CECs detected in early glaucoma with controlled IOP could be attributed only to IOP changes, as eyes with previous surgical and laser procedures known to affect corneal endothelium [26, 27] were excluded from our analyses. The effect of topical antiglaucoma medication on corneal endothelium is negligible according to Wu et al. [20].

Our study revealed a non-significant reduction in mean CEC density in advanced glaucoma when compared to that of healthy eyes and early glaucomatous eyes. This finding is inconsistent with that of Khan et al., who stated that POAG is associated with CEC loss [28, 29]. Verma et al. reported an early reduction in CEC density in patients with ACG, which is attributed to the different nature and course of ACG [13].

In the advanced glaucomatous eyes, we found no correlations between RNFL changes and CEC parameters. This could have been due to the floor effect of marked thinning of the RNFL to a certain level that makes the correlations difficult to detect [30, 31].

We found that the CEC is insulted in POAG, with corneal cell morphology affected in the early stages of POAG, while the cell count was not affected. This conclusion could be helpful from a surgical aspect, especially since most glaucomatous patients would need cataract surgery during different periods of their lives [10, 24]. However, future longitudinal studies with larger sample sizes are needed to verify our results.

Capturing endothelial images via specular microscopy requires that the patients look at a fixation target while sitting with the chin upon a chin rest and the forehead positioned appropriately. The operator displays the pupil and tapes the area around the pupil [32]. The photographing head moves to display the pupil image and

alignment dot on the center of the screen. Then, alignment starts automatically, and photographing is performed. Multiple images should be taken so that a clear, quality image of the central endothelium can be guaranteed [32]. Therefore, for patients with cataracts and early controlled POAG in whom specular microscopy is difficult—such as those who are bedridden or intellectually disabled and patients with neurological diseases associated with head nodding and severe blepharospasm—cataract surgeries could be safely performed based on our finding that the CEC count in eyes with POAG did not differ significantly with that of normal eyes. However, future studies are needed to verify our preliminary results.

Our study had some limitations, as it did not include eyes with ACG for comparison and correlation with healthy eyes and those with early or advanced POAG. Another limitation was our recruitment of eyes only with medically controlled IOP. The inclusion of eyes with treatment-naïve POAG and exposure to high IOP could produce different results, and this should be investigated in future studies. Undoubtedly, longitudinal studies with longer follow-up could provide more conclusive results than a cross-sectional study. The reproducibility of our findings in eyes with medically controlled POAG should be evaluated in a longitudinal study. Future studies with larger sample sizes, longer follow-up durations, and the inclusion of patients with ocular hypertension or different types of glaucoma, such as ACG, alongside those with medically controlled POAG, could provide more robust conclusions to the scientific community.

CONCLUSIONS

CEC parameters were not affected in eyes with early or advanced POAG compared to those of healthy eyes, despite significant RNFL and macular GCC thinning. In the early glaucomatous eyes, a significant correlation was found between morphological characteristics of CECs, such as CV and hexagonality, with superior and inferior RNFL thickness in ONH SD-OCT images. However, in the advanced glaucomatous eyes, no significant correlation was found between RNFL thickness and CEC parameters. This study highlights monitoring the CEC morphological parameters in patients with POAG as an important as following the SD-OCT of the ONH and macular GCC. Following the expected morphological changes of CECs in eyes with early-stage POAG may help in attaining the target IOP that helps not only preserve vision via GCC and RNFL protection, but also helps to protect the corneal integrity.

ETHICAL DECLARATIONS

Ethical approval: The study received ethical approval from the Human Ethical Committee of the Sohag Faculty of Medicine (IRB registration number: Sohag-Med- 22-01-37). Written informed consent was obtained from all participants after an explanation of the study procedure. The tenets of the Helsinki Declaration were followed. **Conflict of interest:** None.

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