Assessment of Corneal Epithelium Thickness in Glaucomatous Patients Undergoing Medical Treatment

Ioannis Halkiadakis, MD, PhD, Anna Vernikou, MD, Vasilis Tsimitis, MD, Ioannis Markopoulos, MD, PhD, Korina Popeskou, MD, and Vasiliki Konstadinidou, MD, PhD

Precis: Patients with glaucoma have reduced and irregular corneal epithelial thickness (CET) even if they do not report symptoms of dry eyes. The reduction of corneal epithelium affects equally the superior and inferior areas of the cornea.

Purpose: To evaluate CET parameters by means of anterior segment optical coherence tomography in glaucomatous patients undergoing medical treatment and compare them with CET parameters of controls.

Methods: This was a cross-sectional study of 62 patients with primary open-angle or pseudoexfoliative glaucoma (POAG group) and 62 age-matched controls. Fourier-domain optical coherence tomography (RTVue) with a corneal adaptor module was used in the present study. The pachymetry scan pattern was used to map the cornea and the software generated corneal thickness parameters were recorded. Simple comparisons between groups were performed and the correlations of CET parameters with parameters associated with medication use (treatment duration, number of medications and number of instillations) were assessed.

Results: Mean age of the patients was 68 ± 11.9 years in the glaucoma group and 65.5 ± 8.5 years in the control group (P = 0.17). Median number of instillations of medication was 2 (range, 1 to 6) for the glaucoma group. Central corneal thickness was 537.6 ± 33.3 μm in the glaucoma group and 550.8 ± 33.7 μm in the control group, respectively (P = 0.028). The central CET was 48.8 ± 3.7 μm in the glaucoma group and 53.5 ± 3.7 μm in the control group (P < 0.001). Similarly, the average superior (2 to 7 mm) CET and the average inferior (2 to 7 mm) CET were almost equally reduced in the glaucoma group. Central corneal thickness was 537.6 ± 33.3 μm in the glaucoma group and 53.5 ± 3.7 μm in the control group (P < 0.001). The central CET was 48.8 ± 3.7 μm in the glaucoma group and 53.5 ± 3.7 μm in the control group (P < 0.001). Similarly, the average superior (2 to 7 mm) CET and the average inferior (2 to 7 mm) CET were almost equally reduced in the glaucoma group (45.7 ± 4 vs. 49.6 ± 3.3 μm, P < 0.001 and 49.7 ± 3.9 vs. 53.5 ± 3.7 μm, P < 0.001, respectively). No CET parameter was correlated with any of the treatment parameters.

Conclusions: Patients treated for glaucoma have uniformly reduced corneal epithelial thickness.

Key Words: glaucoma, intraocular pressure lowering medication, corneal epithelium, central corneal thickness

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Objective: The purpose of the present study was to investigate the CET parameters in patients with glaucoma. In addition, we have tried to investigate if any of the treatment parameters was associated with CET.

METHODS

This was a comparative cross-sectional study of patients with primary open-angle or pseudoxfoliative glaucoma (POAG) and age-matched controls.

In the POAG group we included patients with glaucoma who presented for their regular follow-up visit in the Glaucoma department of the Ophthalmiatrico Eye Hospital of Athens between January 2019 and December 2019. Patients in that group were treated with the same IOP-lowering medication.
medication for at least 1 year before inclusion in the study. In the control group we included aged-matched patients presenting in the outpatient department of Ophthalmiatrie Eye Hospital of Athens for regular examinations during the same period. Exclusion criteria for both groups were any other ocular disease requiring medical treatment, any corneal pathology, ocular surgery within 6 months before inclusion in the study and any other systemic disease affecting ocular surface and tear film such as diabetes mellitus and connective tissue disease. The research followed the tenets of the Declaration of Helsinki. Institutional Review Board (IRB) approval was obtained for the study.

Patients’ Evaluation

Patients included in the study underwent a complete ophthalmic evaluation with ophthalmic and general health history assessment, visual acuity assessment, slit-lamp examination of anterior and posterior segments and IOP measurement. Visual fields and OCT of the retinal nerve fiber layer were also performed. Each subject was asked to complete the Ocular Surface Disease Index (OSDI) questionnaire. From the ophthalmic history we recorded the following parameters regarding topical medication use: the total number of IOP-lowering medications (bottles or minims), the total duration of treatment and the total number of instillations of medications per day.

All measurements were taken in the morning, between 8.00 and 11.00 AM. OCT examination was performed after visual acuity measurement before any other examination.

OCT Examination

Fourier-domain OCT (RTVue; Optovue Inc., Fremont, CA Software Version: 2018, 1, 0, 33) with a corneal adaptor module was used in the present study. The machine worked at 830 nm wavelength and had a scan speed of 26,000 axial scans per second with 5 μm axial resolution. A Pachymetry scan pattern was used to map the cornea. This includes 6 mm lines on 8 meridians centered at the pupil and each line scans 1024 axial points. The software then generates the epithelium boundaries and thickness maps. In this method, the air-tear interface and the epithelium-Bowman layer boundary were identified automatically with a computer algorithm by increased signal intensity at corresponding boundaries. The scan was performed 3 times in each subject. After OCT acquisition, the operator reviewed the quality of the OCT scan. If a poor-quality scan (poor signal, eye lid, or image cropping) was noted, additional scans were taken by the examiner with the same device.

The epithelial thickness map was divided into 4 zones on the basis of diameters: central 2 mm, 2 to 5 mm, 5 to 7 mm, and 7 to 9 mm. Epithelium statistics within the central 7-mm zone, including the average epithelial thicknesses of superior and inferior zones, the minimum and maximum thicknesses and the difference between them (minimum minus maximum), and map SD from the average value of a single epithelial thickness map, were calculated automatically by the RTVue corneal adaptor module software (Fig. 1). To calculate relative central epithelial thickness (RCET) CET was divided by CCT and then multiplied by 100.25

Statistical Analysis

If both eyes were eligible for the study, the right eye of each patient was considered. Kolmogorov-Smirnov test was used to evaluate whether the distributions of numerical variables were normal. The numeric variables were presented as mean ± SD or median (interquartile range). Simple comparisons between groups were performed using either T test or the nonparametric Mann-Whitney U test. The correlations between the different variables were studied accordingly using Pearson r or Spearman ρ correlation coefficient. Multivariate linear regression analysis was performed in the glaucoma group to examine association of CET with age, sex CCT and installations of beta-blockers. The SPSS software (version 18.0.0 for Windows; SPSS Inc., Chicago, IL) was used for all statistical analyses. Values of P < 0.05 were considered to indicate statistical significance.

RESULTS

A total of 124 patients were included in the study, 62 in the glaucoma group and 62 in the control group. Mean age of the patients in the glaucoma group was 68.1 ± 11.9 (35 to 90) patients in the glaucoma group and 65.5 ± 8.6 (44 to 81) in the control group, respectively (P = 0.17). The demographics and the clinical characteristics of the study population are shown in Table 1.

Patients with glaucoma had reduced corneal thickness parameters. CCT was 537.6 ± 33.3 μm in the glaucoma group and 550.8 ± 33.7 μm in the control group, respectively, P = 0.028. Minimum corneal thickness was as well reduced in the glaucoma group (P = 0.06). Furthermore, central corneal stromal thickness was reduced in the glaucoma group, but the difference did not reach statistical significance (Table 2).

Central CET was 48.7 ± 4.1 μm in the glaucoma group and 54.3 ± 3.7 μm in the control group, respectively (P < 0.001). The epithelium thickness was higher in the inferior zone in both groups, but the thickness of the epithelium was almost equally reduced in the superior (2 to 7 mm) (4.8 ± 0.7 μm) as well as in the inferior zones (2 to 7 mm) (4.5 ± 0.8 μm) in the glaucoma patients. Although the SD of CET was higher in patients with glaucoma this difference did not reach statistical significance. Glaucomatous eyes, however, displayed an increased difference between maximum and minimum thickness (14 vs. 12 μm, respectively, P = 0.04). Regarding type of medication central CET was reduced in patients receiving beta-blockers (Table 3). CET was not significantly correlated with the severity of glaucomatous pathology, expressed with the visual field index (Spearman ρ = 0.058, P = 0.69).

In both groups, central CET was not found to have a statistically significant correlation with the age of the patients or the OSDI score (Table 4). In the glaucoma group, no statistically significant correlation of central CET or RCET with the number of medications used (Spearman ρ = −0.008, P = 0.44 and 0.17, P = 0.2, respectively), the total duration of treatment (Spearman ρ = −0.19, P = 0.14 and 0.6, P = 0.6, respectively), the total number of instillations per day (Spearman ρ = −0.16, P = 0.3 and 0.07, P = 0.6, respectively) and the total number of administered installations (Spearman ρ = −0.205, P = 0.110) (Table 4). No significant association was observed between central CET and CCT in the control group (Pearson r = 0.169, P = 0.11) or in the group of glaucoma patients (Pearson r = 0.203, P = 0.11). The results of the multivariate linear regression analysis in the glaucoma patient group documented the inverse association with installations of beta-blockers (P = 0.006), whereas an inverse association with age (P = 0.040) emerged (Table 5).
DISCUSSION

The present study examined OCT generated corneal thickness maps of patients with glaucoma and aged-matched normal volunteers and made comparisons between them. A small but statistically significant reduction of CET was verified in glaucoma patients that uniformly affected all regions of the cornea. This is in accordance with a very recent study by Doğan et al. They compared CET of patients using antiglaucomatous medication with that controls and reported that the median central CET was significantly reduced in patients under treatment (56 vs. 60 μm, \( P < 0.001 \)). It is of note that in their study only the central CET was measured and the measurements were done manually using calipers. The present study compared the measurements generated by the epithelial thickness mapping software. Therefore, apart from central CET comparisons were made in average thickness of the superior and inferior epithelial zones. Surprisingly the magnitude of the reduction in epithelial thickness was almost equal the superior and the inferior zones. Centamo et al\(^{24}\) using the same OCT device with us reported statistically significant difference between controls and patients with early corneal damage from antiglaucomatous medication in the central CET (56.58 vs. 51.38 mm). However, this deference was not present in patients with advanced corneal damage. The authors hypothesized that in later stages of corneal damage corneal epithelial subedema and early inflammatory cell infiltration resulted in an increase in CET. Contrary to the results of the present study, Franzoz et al\(^{23}\) reported no difference in central CET in dry eyes and in eyes chronically treated for glaucoma or ocular hypertension with IOP-lowering eye drops. However, the measurements were done manually and no information of the characteristics of their patient population is provided. Batawi et al\(^{25}\) used calipers to measure central CET in OCT derived images of male patients with glaucoma. Although glaucoma patients had reduced central CET the difference between glaucoma patients and controls was marginal (45.8 vs. 46.9 μm, \( P = 0.1 \)). Nevertheless, the same study reported reduced corneal stromal thickness in glaucoma patients. The present study did not find any significant difference between male and female glaucoma patients. Therefore, the different results of the present study may be attributed to differences in the patient population concerning the race (only white patients participated in the present study), the number of medications, the comorbidities (diabetics were excluded in the present study), as well as to different measurement techniques (manual vs. automatic).

The present study reported an increased difference of maximum and minimum epithelial thickness in the glaucoma population. To our understanding this may indicate an increased irregularity of corneal epithelium in the glaucoma patients. It is of note that the central CET was significantly reduced in patients under treatment (56 vs. 60 μm, \( P < 0.001 \)). The measurements were done manually using calipers. The present study compared the measurements generated by the epithelial thickness mapping software. Therefore, apart from central CET comparisons were made in average thickness of the superior and inferior epithelial zones. Surprisingly the magnitude of the reduction in epithelial thickness was almost equal the superior and the inferior zones. Centamo et al\(^{24}\) using the same OCT device with us reported statistically significant difference between controls and patients with early corneal damage from antiglaucomatous medication in the central CET (56.58 vs. 51.38 mm). However, this deference was not present in patients with advanced corneal damage. The authors hypothesized that in later stages of corneal damage corneal epithelial subedema and early inflammatory cell infiltration resulted in an increase in CET. Contrary to the results of the present study, Franzoz et al\(^{23}\) reported no difference in central CET in dry eyes and in eyes chronically treated for glaucoma or ocular hypertension with IOP-lowering eye drops. However, the measurements were done manually and no information of the characteristics of their patient population is provided. Batawi et al\(^{25}\) used calipers to measure central CET in OCT derived images of male patients with glaucoma. Although glaucoma patients had reduced central CET the difference between glaucoma patients and controls was marginal (45.8 vs. 46.9 μm, \( P = 0.1 \)). Nevertheless, the same study reported reduced corneal stromal thickness in glaucoma patients. The present study did not find any significant difference between male and female glaucoma patients. Therefore, the different results of the present study may be attributed to differences in the patient population concerning the race (only white patients participated in the present study), the number of medications, the comorbidities (diabetics were excluded in the present study), as well as to different measurement techniques (manual vs. automatic).

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population. A recent study using an ultrahigh-resolution optical coherence tomography found that patients with DES have irregular ocular surface while normal subjects have a smoother surface. The authors presumed that this irregularity is most probably a manifestation of the injurious effect of dryness on the ocular surface. The same principle may apply regarding the deleterious effect of glaucoma drops. There are many possible causes that alone or in combination may account for the thickness reduction and irregularity of corneal epithelium: CET changes may be intrinsic to the pathology of glaucoma, a result of chronic topical therapy with preservatives shown to be toxic to ocular surface, or a consequence of the active ingredients in the medications. Our patient population consisted of glaucoma patients receiving multiple treatments for many years. Most of the glaucoma medication available in Greece contained BAK as preservative. One of the main causes of the thickness reduction and irregularity of corneal epithilum is BAK toxicity. BAK has been demonstrated to have adverse effects on the cornea and conjunctiva and it may induce cell toxicity and ocular damage in a dose-dependent manner. Evidence from animal studies and human tissue cell culture experiments, suggests that BAK can cause detrimental effects on the superficial ocular tissue. These findings are supported by evidence from clinical studies. Martone et al using in vivo confocal microscopy to evaluate ocular surface reported reduced density of superficial (10 to 15 μm) corneal epithelial cells in all groups of glaucoma patients, except the preservative-free group that according to the authors, could be related to the toxic effects of BAK. Three mechanisms of BAK toxicity have been described: a detergent effect, causing loss of tear film stability; direct damage to the corneal and conjunctival epithelium; and immunological reaction. BAK is a quaternary ammonium that acts as a detergent on the lipid layer of the tear film, reducing stability, increasing evaporation rate, and thus increasing tear osmolarity. Exposure to tear film hyperosmolarity causes osmotic stress for the surface epithelium generating apoptosis.

The present study found that the thickness of corneal epithelium was almost equally reduced in the superior as well as in the inferior zones of POAG patents. This is in contrast with DES patients who according to Cui et al exhibit CET reduction in the superior zones. This finding implies different mechanisms of injury between the 2 entities. Direct toxicity to cells instead of only the occurrence of DES might be the cause of this difference in our patient population with POAG.

The design of the present study does not allow us to reject the hypothesis that the disease itself is associated with
reduced CET. However, Fogagnolo et al.38 after studying with in vivo confocal microscopy corneas of treated and untreated glaucomatous concluded that healthy controls alongside with newly diagnosed untreated glaucomatous eyes presented similar values on all studied confocal parameters, while the same confocal parameters of treated glaucomatous eyes were significantly different. Future studies could further elucidate the matter, by recruiting alternative control groups on treatment-naive glaucoma patients or patients on chronic topical therapy for other ocular conditions excluding glaucoma.

The present study did not find any association between CET parameters and OSDI score. This is not surprising. Previous studies have indicated week correlation of symptoms and signs of tear film dysfunction in DES patients39,40 as well as in patients with glaucoma.41 It is noteworthy, however, that the reduction of the CET occurred despite the fact that only 25% of glaucoma patients were symptomatic (OSDI>12). Furthermore, the present study did not find any correlation between CET and the parameters of glaucoma treatment. Dogan et al.26 reported that there was no significant difference in central CET, in terms of glaucoma type, duration of therapy, the number of drugs, and the number of daily drops. Contrary to this finding Batawi et al.25 reported that the number of glaucoma medications remained significantly correlated with CET. It is likely that differences in the number of glaucoma medication, type of preservative (BAK or newer preservatives such as Polysural) as well as degree of compliance of participants among the different studies may account for the different results.

The present study examined if there was a difference in central CET in patients receiving different glaucoma medication. The only beta-blocker in our patient population was timolol. Patients receiving timolol in their treatment regimen were excluded. In addition to this 25% of patients were receiving preservative-free medication. The explanation might be that topical timolol treatment, acting also in a systemic way, was responsible of a reduction in tear production affecting even more CET.42,43

There are several limitations in the present study. The major limitation was the great variability in the treatment regimen of the study participants. Twenty-eight participants were receiving 2 or more medications and more than half were receiving fixed combinations. This fact prevents us from drawing safe conclusions regarding the exact effect of each medication on CET. Another limitation is that is until today there is no secure way to verify the adherence of patients to the treatment regimen. Adherence in general is not optimal. Furthermore, it has been shown that the adherence is diminishing with the increasing the number of medication.44,45

In conclusion the present study has shown that patients with glaucoma have reduced CET in comparison with controls. This reduction is observed in the superior as well as in the inferior part of the cornea. The present study implied and increased irregularity of CET of glaucoma patients in a similar manner that is observed in dry eye patients.

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### TABLE 4. Correlation Between CET and Demographics and Treatment Parameters

| Variables                | Pearson r/Spearman ρ | P       |
|--------------------------|-----------------------|---------|
| Age (y)                  | −0.18                 | 0.85    |
| OSDI                     | −0.04                 | 0.70    |
| No. meds                 | −0.27                 | 0.04    |
| Instillations            | −0.27                 | 0.04    |
| Total number of instillations | −0.36           | 0.01    |
| Years of treatment       | −0.38                 | 0.01    |

CCT indicates central corneal thickness; CET, central corneal epithelial thickness; OSDI, Ocular Surface Disease Index; RCET, relative central epithelial thickness.

### TABLE 5. Results of the Multivariate Regression Analysis Examining Independent Associations With Central Corneal Epithelial Thickness in the Glaucoma Patient Group

| Variables | Beta Coefficient (95% CI) | P       |
|-----------|---------------------------|---------|
| CCT       | +0.02 (−0.01 to +0.04)     | 0.228   |
| Beta-blockers vs. no | −2.56 (−4.36 to −0.75)     | 0.006   |
| Age       | −0.08 (−0.16 to −0.004)    | 0.040   |
| Sex       | −0.37 (−2.27 to +1.53)     | 0.700   |

CCT indicates central corneal thickness; CI, confidence interval.
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