Anterior-Segment Optical Coherence Tomography and Scanning Electron Microscopy to Evaluate Corneal Epithelial Changes in Patients Undergoing Glaucoma Therapy

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Purpose: To measure corneal epithelial thickness (CET) in patients with glaucoma using anterior-segment optical coherence tomography and to evaluate CET changes in relation to corneal epithelial microvilli analyzed by scanning electron microscopy (SEM).

Methods: Twenty-two eyes (16 patients) being treated with preservative-containing topical medications and 12 normal eyes underwent anterior-segment optical coherence tomography imaging using RTVue-100. The CET maps generated corresponded to a 6-mm diameter area of cornea that was divided into 17 sectors. We compared the CETs of each sector obtained in the glaucomatous group with those obtained in the control group.

Results: Glaucomatous eyes were divided into 2 groups based on the number of microvilli on SEM: group 1 (6 eyes) = grades 1 and 2 at SEM (range: 500–3000) and group 2 (10 eyes) = grades 3 and 4 at SEM (range: 0–500). Four CET sectors were significantly thinner in group 1 than in normal eyes: central (P = 0.012), superior (P = 0.005), temporal paracentral (P = 0.003), and temporal midperipheral (P = 0.023). No significant differences were observed between group 2 and normal eyes. CET sectors were significantly thinner in group 1 than in group 2 only in the superior (P = 0.024) and superior-temporal paracentral (P = 0.020) sectors. CET progressively increased in patients with glaucoma as the number of corneal epithelial microvilli decreased.

Conclusions: CET and corneal epithelial microvilli are new parameters with which to evaluate early stages of corneal epithelial changes during glaucoma therapy. In advanced stages of corneal epithelial damage, SEM evaluation reveals ultrastructural epithelial changes that may not be observed on CET measurements.

Key Words: corneal epithelial thickness, corneal epithelial microvilli, anterior-segment optical coherence tomography, scanning electron microscopy, antiglaucoma drugs

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The chronic use of antiglaucomatous therapy can induce alterations in the ocular surface, particularly the corneal epithelium.[1–4] Changes in the corneal epithelium are usually evaluated using scanning electron microscopy (SEM) applied to corneal impression cytology that provides a reproducible evaluation of the microstructure of corneal epithelial microvilli.[5–7] Damage of the ocular surface is now also evaluated based on corneal epithelial thickness (CET) because previous techniques generally used to measure CET, namely, high-frequency ultrasound and confocal microscopy, were invasive and required the use of topical anesthesia and, moreover, had several sources of error.[8–10] Thanks to the advent of the noninvasive imaging technique, anterior-segment optical coherence tomography (AS-OCT), it became possible to monitor CET.[11–13] This fast noncontact tool generates a CET map divided into sectors.[14–16] It is important to study the CET and microvilli in patients with glaucoma because their modifications are associated with the development of the ocular surface disease consequent to the prolonged use of glaucoma therapy.[17–19] The purpose of this retrospective study was to evaluate anatomical ultrastructural changes of the corneal epithelium using AS-OCT and SEM applied to impression cytology in patients undergoing chronic topical glaucoma therapy.

MATERIALS AND METHODS

In this retrospective study, we evaluated 22 consecutive eyes of 16 patients affected by open-angle glaucoma enrolled from February to April 2017 in the Eye Clinic of the University of Naples "Federico II." Each patient underwent evaluation of best-corrected visual acuity according to the Early Treatment of Diabetic Retinopathy Study, using Goldman applanation tonometry, gonioscopy, slit-lamp biomicroscopy, fundus examination with a +90 D lens, standard visual field testing (perimetry), and spectral domain (SD)-OCT.[20]

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All patients were on preservative-containing topical medications and the mean duration of treatment was 10 years.

The cornea of each patient was evaluated by slit-lamp biomicroscopy, the Fourier-Domain AS-OCT system, and SEM applied to impression cytology. Twelve normal eyes of 6 individuals (1 male and 5 female patients, mean age 74 ± 1 years) with normal ophthalmic examination results, intraocular pressure below 21 mm Hg, normal visual field tests, and without a family history of glaucoma served as the control group. The exclusion criteria were current or past ocular disease, surgery, or trauma; dry eye disorder; history of contact lens wear; and ocular or systemic diseases that may affect the cornea.

The study was approved by our Institutional Review Board and informed consent was obtained from the patients enrolled in the study. All procedures involving patients were in accordance with the ethical standards of our Institutional Review Board and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The primary outcome measure of this study was the reliability of AS-OCT in measuring CET in patients undergoing glaucomatous therapy and the secondary outcome was to evaluate changes in CET based on the number of corneal microvilli measured at SEM.

AS-OCT System

The Fourier-Domain AS-OCT system RTVue (Optovue Inc, Fremont, CA) with a cornea anterior module long adapter lens and software version A6 (9.0.27) was used to measure CET. Data output included CET maps corresponding to an area 6 mm in diameter of the central cornea. The settings were L-Cam lens, 8 meridional B-scan per acquisition, consisting of 1024 A-scans, each with an axial resolution of 5 μm. Each pachymetry map was divided into 17 sectors: a central zone 0 to 2 mm in diameter, 8 paracentral zones 2 to 5 mm in diameter, and 8 peripheral zones 5 to 6 mm in diameter. AS-OCT also measures minimum (Min) CET, maximum (Max) CET, Min–Max (CET difference of Min and Max), and SD (topographic variability of CET).[16]

Impression Cytology and SEM

After CET evaluation, an impression cytology specimen was obtained from each eye for SEM. Corneal epithelium specimens were collected by pressing a fragment of cellulose acetate on the corneal surface for 3 or 4 seconds. Anesthetic was not used during this procedure so as not to affect the microvilli analysis; no patient complained of ocular discomfort. The corneal specimens were transferred to a glass slide by pressing the cellulose acetate fragment on the glass slide for 30 seconds and fixed in 3% glutaraldehyde in 0.065 M (pH 7.4) phosphate buffer for 2 hours at room temperature. The slides were washed 3 times in 0.065 M phosphate buffer (for 30 minutes) and then placed in 1% OsO4 in 0.064 M (pH 7.4) phosphate buffer for 30 minutes. The samples were dehydrated through a graded series of ethanol and then critical-point-dried in a CO_2 liquid Model SPS-1500 apparatus (Bomar, Co, Tacoma, WA). The specimens were mounted on aluminum stubs with silver-conducting paint, sputtered with a thin (20-nm) gold film, and observed with a Cambridge Mark 250 scanning electron microscope.[5]

Microvilli Evaluation

Microvilli were counted with the aid of a magnifier and graticule on photographic prints. The microvilli on each sample were first identified at ×750 in a 1500 μm^2 field. Then, at ×7500, the number of microvilli in each 230 μm^2 area of the field selected was counted, and the mean number of microvilli and SD were calculated. Based on the number of microvilli, specimens were classified as grade 1 (n = 1500–3000), grade 2 (n = 500–1500), grade 3 (n = 100–500), and grade 4 (n = 0–100).[6] Grades 1 and 2 corresponded to early and mild alteration of the ocular surface, respectively, and grades 3 and 4 to moderate and severe alteration, respectively. Group 1 consisted of eyes that presented corneal microvilli classified as grades 1 and 2 on SEM, whereas group 2 consisted of eyes with corneal microvilli classified as grades 3 and 4 on SEM.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (version 20.0 for Windows; SPSS Inc, Chicago, IL). One-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis was used to evaluate differences in each CET sector between the glaucomatous and control groups. A P value of <0.05 was considered statistically significant.

RESULTS

Sixteen patients with glaucoma (5 female and 11 male patients, mean age 71 ± 1 years), for a total of 22 eyes, were enrolled in this retrospective study. The control group was constituted by 12 eyes of 6 individuals (1 male and 5 female patients, mean age 74 ± 1 years). No significant age differences were found between patients and controls. The CET values of glaucomatous eyes were divided into 2 groups based on the degree of alteration of corneal microvilli measured at SEM. Group 1 consisted of 6 eyes with corneal microvilli classified as grades 1 and 2 at SEM (range n = 500–3000), whereas group 2 consisted of 10 eyes with corneal microvilli classified as grades 3 and 4 at SEM (range n = 0–500). The mean duration of glaucoma therapy was 9 ± 2 years (range 8–10 years) and 12 ± 1 years (range 10–14 years) in groups 1 and 2, respectively. Table 1 lists the demographic and clinical information of the whole group and of each single group. Figure 1 shows representative examples of CET measurements and corneal epithelial microvilli evaluation on AS-OCT and SEM, respectively, in normal eyes and in glaucomatous eyes of groups 1 and 2. As shown in Table 2, ANOVA revealed significant differences in central, superior, and temporal CET sectors between the glaucomatous and control groups. Bonferroni post hoc analysis showed statistically significant differences ($P < 0.005$) between controls and group 1 in the central (56.58 ± 3.63 vs. 51.38 ± 3.50 μm),

TABLE 1. Demographic and Clinical Data of All Patients and of Groups 1 and 2

	Total No. of Patients	Group 1 Patients	Group 2 Patients
Eyes (n)	16	6	10
Age (yr)	71 ± 13	78 ± 10	67 ± 14
Gender (male/female)	11/5	3/3	8/2
Type of therapy			
Monotherapy (n. eyes)	10	4	6
Multi-therapy (n. eyes)	6	2	4
Duration of therapy (yr)	10 ± 2	9 ± 2	12 ± 1
IOP (mm Hg)	15.7 ± 0.5	15.3 ± 2.5	16.1 ± 1.4

Data are expressed as mean ± SD.
IOP, intraocular pressure.

superior (53.33 ± 3.93 vs. 47.13 ± 3.31 μm), temporal paracentral (54.92 ± 3.32 vs. 48.88 ± 3.40 μm), and temporal midperipheral (53.17 ± 2.86 vs. 48.38 ± 3.07 μm) sectors. On the contrary, no significant CET differences were found between controls and group 2 ($P > 0.005$). CET sectors were significantly thinner in group 1 than in group 2 only in the superior (47.13 ± 3.31 vs. 52.07 ± 4.60 μm) and superior-temporal (46.88 ± 3.31 vs. 51.93 ± 4.55 μm) paracentral sectors.

DISCUSSION

To our knowledge, this retrospective study is the first report of CET measurements in glaucomatous eyes divided into 2 groups based on the number of corneal epithelial microvilli identified at SEM. We found that the CET in the central, superior, and temporal areas of the paracentral and midperipheral sectors was significantly thinner in the glaucomatous group 1 than in the control group. This thinning of CET could result from the progressive cellular damage that initially involves corneal epithelial microvilli in patients undergoing glaucoma therapy.[6] The apical membrane of the superficial epithelial cells is covered by microvilli, which are themselves covered by glycocalyx, the deepest layer of the tear film.[21–23] Prolonged use of topical preservative-containing antiglaucoma therapy can derange tear film stability.[17,18] Alteration of the tear film damages the structure of corneal epithelial microvilli.[24–27] The reduced CET in superior sectors in glaucomatous group 1 could be explained by the eyelid dynamics that, during blinking, can chafe the epithelium with greater forces applied on the upper eyelid than on the lower eyelid,[28] whereas the increased CET in inferior sectors in this group could be related to the upright sitting position during the examination that may contribute to a thicker inferior tear film because of gravity.[16]

Although patients in glaucomatous group 2 had a greater reduction in microvilli than group 1, their CET did not differ

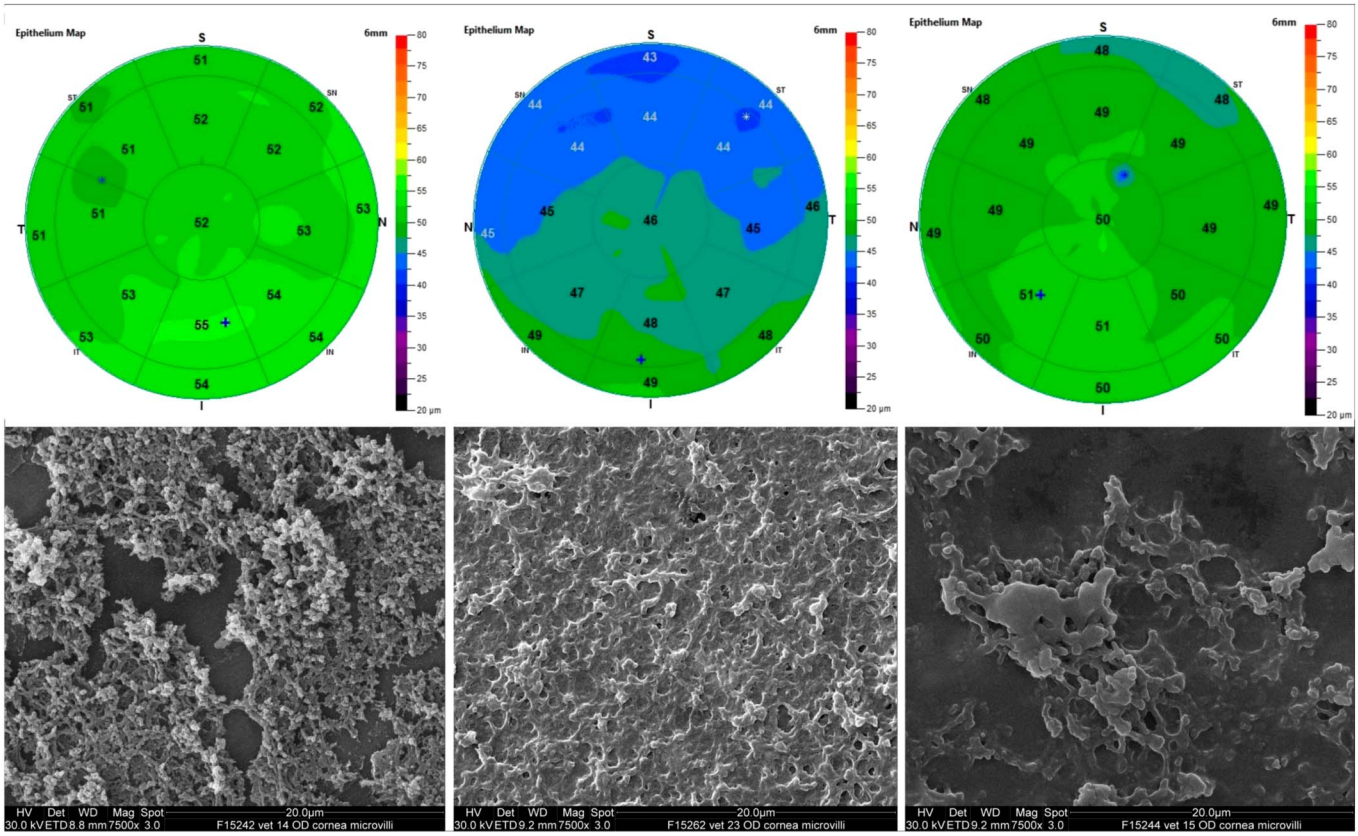

FIGURE 1. Representative corneal epithelial map (top row) and corresponding corneal epithelial microvilli evaluation (bottom row) in normal eyes (left panel), glaucomatous eyes group 1 (middle panel), and glaucomatous eye group 2 (right panel).

TABLE 2. CET Parameters in Patients With Glaucoma Divided According to the Degree of Alteration of Corneal Microvilli Measured by SEM

	Control	Group 1	Group 2	Control Versus Group 1, *P*	Control Versus Group 2, *P*	Group 1 Versus Group 2, *P*	ANOVA
Central CET, μm	56.58 ± 3.63	51.38 ± 3.5	54.79 ± 4.23	0.018	0.738	0.166	0.021
Paracentral CET, μm							
Superior sector	53.33 ± 3.93	47.13 ± 3.31	52.07 ± 4.6	0.005	1.000	0.024	0.005
Superior nasal sector	53.83 ± 2.82	49 ± 3.7	52.79 ± 4.87	0.037	1.000	0.120	0.035
Superior temporal sector	53.42 ± 3.42	46.88 ± 3.31	51.93 ± 4.55	0.003	1.000	0.020	0.003
Inferior sector	54.25 ± 2.93	52.13 ± 2.36	55.36 ± 5.12	0.729	1.000	0.215	0.192
Inferior nasal sector	54 ± 3.1	52.38 ± 2.5	55 ± 5.1	1.000	1.000	0.437	0.341
Inferior temporal sector	54.75 ± 2.99	51.63 ± 3.29	53.71 ± 6.02	0.430	1.000	0.928	0.334
Nasal sector	54.17 ± 2.59	51.5 ± 3.89	53.86 ± 4.96	0.467	1.000	0.584	0.313
Temporal sector	54.92 ± 3.32	48.88 ± 3.4	52.5 ± 3.92	0.003	0.293	0.090	0.004
Minimum	50.08 ± 3.2	44.38 ± 3.11	49.14 ± 5.01	0.012	1.000	0.037	0.011
Maximum	58.5 ± 3.55	54.88 ± 3.1	57.29 ± 4.63	0.160	1.000	0.536	0.148
Min–Max	8.42 ± 3.26	10 ± 4.28	8.14 ± 2.8	0.923	1.000	0.659	0.441
SD	1.85 ± 0.74	2.71 ± 1.03	1.9 ± 0.64	0.063	1.000	0.074	0.041
Midperipheral CET, μm							
Superior sector	50.83 ± 3.86	45.75 ± 2.91	50.71 ± 5.68	0.061	1.000	0.059	0.036
Superior nasal sector	52.67 ± 2.5	47.75 ± 3.2	51.93 ± 5.73	0.051	1.000	0.105	0.042
Superior temporal sector	51.25 ± 3.62	46.25 ± 3.06	50.36 ± 6.11	0.082	1.000	0.178	0.070
Inferior sector	53.5 ± 3.55	51.38 ± 2.97	55.07 ± 5.98	0.969	1.000	0.245	0.213
Inferior nasal sector	53.08 ± 2.78	51 ± 1.92	54.5 ± 5.6	0.819	1.000	0.188	0.171
Inferior temporal sector	54.08 ± 3.70	51.5 ± 3.34	54.78 ± 5.78	0.691	1.000	0.357	0.280
Nasal sector	53.67 ± 2.46	50.38 ± 2.97	53.14 ± 4.94	0.200	1.000	0.330	0.154
Temporal sector	53.17 ± 2.86	48.38 ± 3.07	51.5 ± 4.5	0.023	0.774	0.193	0.032

Data are expressed as mean ± SD.
Statistical significance $P < 0.05$.

from that of controls. These results show that in patients treated with preservative-containing antiglaucoma medications, CET progressively increased as the number of corneal epithelial microvilli decreased. We hypothesize that the initial reduction of number of microvilli that results in CET thinning is followed by corneal changes, namely, corneal epithelial subedema and early inflammatory cell infiltration that determines an increase in CET. Corneal epithelial cells, exposed to hyperosmotic conditions resulting from antiglaucoma therapy, progressively degenerate as witnessed by the appearance of irregular cell shapes, loss of cell borders, and disruption of intercellular connections.[27,29,30] Alteration of tight junctions affects the barrier integrity of the corneal epithelium, thereby causing subedema of corneal epithelial cells and early inflammatory cell infiltration.[2,31–33] In fact, an inflamed ocular surface is closely related to an increase of dendritic cells that modulate the immune response in the corneal epithelial layer.[31–38] The presence of inflammation in the corneal epithelium could explain the increased CET in our patients with glaucoma who presented advanced damage of epithelial microvilli on SEM.

AS-OCT and SEM are 2 techniques that together could be useful to evaluate corneal epithelial structures that play an important role in the management of the ocular surface in patients with glaucoma.[5–7,11–13] Corneal epithelial microvilli and CET are 2 indicators of early epithelial damage during chronic antiglaucoma therapy. In advanced stages of corneal epithelial damage, SEM evaluation reveals ultrastructural epithelial changes that may not appear at CET measurements. The limitation of this study is the relatively small sample size of the groups of patients which precluded evaluation of the role of diverse types of antiglaucoma therapy in corneal epithelial damage. Moreover, studies are needed to evaluate CET and microvilli changes related to treatment duration. In addition, it would be interesting to compare glaucoma therapy with and without preservatives to understand the role of preservatives in changes of the ocular surface. Another issue to address is the possible correlation between CET and microvilli alterations with ocular surface clinical tests such as break-up time, fluorescein staining, the Schirmer test, and the Ocular Surface Disease Index questionnaire score.[39–41]

In conclusion, CET, measured using AS-OCT, is a new parameter with which to evaluate the ocular surface in the early stages of corneal epithelial changes during glaucoma therapy. In advanced stages of corneal epithelial damage, it is also necessary to use SEM to obtain additional information on the anatomical ultrastructure of the ocular surface.

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