Ocular Surface Epithelial Thickness Evaluation with Spectral-Domain Optical Coherence Tomography

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PURPOSE. To use in vivo spectral-domain optical coherence tomography (SD-OCT) to measure corneal, limbal, and bulbar conjunctival epithelial thickness.

METHODS. A total of 156 eyes of 79 subjects were enrolled in four groups: young control (YC group), < 40 years; middle-aged control (MAC group), 40–49 years; patients with dry eye syndrome (KCS group); and patients with glaucoma or ocular hypertension treated with intraocular pressure (IOP)-lowering medication (G group). The central corneal epithelium (CE) thickness, and the limbal (LE) and bulbar conjunctival epithelium (BCE) thickness in four quadrants were measured using SD-OCT.

RESULTS. The mean thicknesses of the CE, LE, and BCE of the YC group were 48.5 ± 2.9 μm, 83.0 ± 14.3 μm, and 42.0 ± 7.5 μm, and for the MAC group were 48.8 ± 3.0 μm, 84.5 ± 10.1 μm, and 42.2 ± 7.9 μm, respectively. The ocular surface epithelial thickness was not significantly different between the YC and MAC groups. CE thicknesses were not significantly different between the KCS, G, and control groups. The mean LE thicknesses were significantly lower in the KCS and G groups compared with the MAC group. The mean BCE was significantly thicker in the KCS and G groups compared with the MAC group.

CONCLUSIONS. Anterior segment SD-OCT can provide a noninvasive evaluation of ocular surface epithelial thickness. LE and BCE thickness was modified in dry eye patients and patients using IOP-lowering eye drops, whereas aging seemed to have no effect. (Invest Ophthalmol Vis Sci. 2011;52:9116–9125)

PATIENTS AND METHODS

Subjects

A total of 156 eyes of 79 subjects were consecutively enrolled from July to November 2010. This study was performed at the Center of Clinical Investigations (CIC 503) at the Quinze-Vingts National Ophthalmology Hospital, with the approval of the Institutional Review Board of Saint-Antoine University Hospital (CPP-Ile de France 5, number 10,793), and in accord with the Declaration of Helsinki. All subjects were informed of the aims of the study, and their consent was obtained.

Fifty-five eyes of 28 healthy volunteers were enrolled. The young control (YC) group comprised subjects aged < 40 years (n = 18 subjects; nine women and nine men; mean age 28.5 ± 6.5 years; range 20 to 39 years). The middle-aged control (MAC) group comprised subjects aged 40–49 years (n = 10 subjects; four women and six men; mean age 43.3 ± 8.6 years; range 41 to 66 years). All healthy volunteers (YC and MAC groups) had no complaint of ocular surface irritation, and no anterior segment abnormality on biomicroscopic examination and ocular surface tests. Fifty-six eyes of 28 patients with dry eye syndrome (keratoconjunctivitis sicca [KCS] group) were enrolled. The KCS group comprised three patients with Sjögren syndrome dry eye and 25 patients with non-Sjögren syndrome dry eye (24 women and four men; mean age 58.5 ± 13.7 years; range 26 to 81 years). Dry eye was defined as a Schirmer 1 testing < 10 mm, interpupillary ocular surface fluorescence staining (at least 2 on the Oxford scheme), and tear film instability accompanied by complaints of ocular irritation.
five eyes of 23 patients with glaucoma or ocular hypertension treated with intraocular pressure (IOP)-lowering medications were enrolled. The glaucoma group (G) comprised 13 women and 10 men with a mean age of 53.9 ± 14.0 years (range 25 to 78 years). Exclusion criteria for all groups were: age < 18 years, subject unable to complete the questionnaire or understand the procedures, current or use within the last 6 months of eye drops (other than nonpreserved tear substitutes for the KCS group, and antiglaucoma medications for the G group), a change in the antiglaucomatous topical regimen within the last 6 months, previous eye surgery, contact lens wear, and a local or systemic disease or treatment that could influence the ocular surface.

Ophthalmologic Examination

Demographic information and medical history was obtained from the patients’ medical records. Each subject was asked to complete the Ocular Surface Disease Index (OSDI) questionnaire. Then, all patients underwent a complete examination of the ocular surface in both eyes in the following order: tear film break-up time (TBUT), corneal and conjunctival fluorescein staining using the Oxford scheme, and Schirmer test without anesthesia. Finally, according to the 2007 Dry Eye Workshop, a severity grading scheme (1–4) was used to evaluate symptoms and signs of ocular surface disease.

OCT Examination and Image Analysis

An SD-OCT fitted with an anterior segment module (Spectralis OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) was used. The OCT axial and lateral optical resolutions were 3.9 μm and 11 μm, respectively. All acquisitions were taken using the high-resolution mode with an acquisition time of 19 ms per image. The instrument combines OCT technology with a confocal scanning laser ophthalmoscope (cSLO) to provide a live view of the eye to control the location of the OCT scan. Because OCT is a noncontact technique, it was performed before the ophthalmologic examination and ocular surface tests to avoid potential artifacts. For each eye, one image of the central cornea and one image of the limbo-conjunctival area in each quadrant (superior, inferior, temporal, and nasal) were analyzed. A horizontal scan was used for imaging the central cornea, and the nasal and temporal limbus and conjunctival areas, whereas a vertical scan was used for the inferior and superior limbus and conjunctival areas.

The images were analyzed with a zoom factor of 600% to 800% provided by the SD-OCT software (Spectralis OCT; Heidelberg Engineering GmbH) with a resolution of 3.87 μm/pixel axially and 11.1 μm/pixel laterally.

The central corneal epithelium (CE) thickness, the limbal epithelium (LE) thickness, and the bulbar conjunctival epithelial (BCE) thickness, between 2 and 3 mm from the limbus of each quadrant, were measured using the cursors provided by the SD-OCT software (Fig. 1). The cursors were placed perpendicular to the ocular surface epithelium from a point located just beneath the tear film (first hyperreflective layer) to the basal membrane (second hyperreflective layer). For each quadrant, three measures were obtained, and results were expressed as mean ± SD. All measurements were made by one examiner (MF) who was masked to the subject’s ophthalmologic examination and status. To evaluate interobserver variability, a second examiner (IK) who was masked to the first measurements, evaluated the CE, LE, and BCE from the same images obtained on 19 randomized eyes. A Wilcoxon paired test demonstrated no significant differences between the two examiners.

Statistical Analysis

Results for the descriptive statistics are presented as the mean ± SD. Simple comparisons between groups were performed using the nonparametric Mann-Whitney U test, and comparisons between several groups were performed using the Kruskal-Wallis test. The correlations between the different variables were studied using Spearman’s correlation coefficient. Probability values < 0.05 were considered signifi-

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933459/ on 03/31/2018)
cant. All statistical analyses were performed using statistical software (XLSTAT 2010; Addinsoft, Paris, France).

RESULTS

Corneal, Limbal, and Conjunctival Epithelia in Healthy Eyes

Between Group Comparisons. The mean thicknesses of the CE, LE, and BCE of the YC group were 48.3 ± 2.9 μm, 83.0 ± 14.3 μm, and 42.0 ± 7.5 μm, and for the MAC group 48.8 ± 3.0 μm, 84.3 ± 10.1 μm, and 42.2 ± 7.9 μm, respectively. In both groups, the thickest epithelium was the LE followed by the CE (**P < 0.0001 in both groups, comparing LE with CE) and BCE (*P = 0.005 in YC and **P = 0.019 in MAC comparing BCE with CE). There was no statistical difference in CE, LE, and BCE thickness between the YC and MAC groups.

Similarly, no statistical correlations between CE, LE, or BCE and age were observed. Results are presented in Figure 2 and Table 1.

Quadrant Comparisons. In both control groups, the LE in the superior and inferior quadrants (95.6 ± 15.0 μm and 98.0 ± 23.7 μm in YC; 97.2 ± 11.1 μm and 106.8 ± 20.0 μm in MAC; respectively) were thicker than in the nasal and temporal quadrants (67.9 ± 10.7 μm and 69.3 ± 14.5 μm in YC; 67.9 ± 7.5 μm and 66.9 ± 11.9 μm in MAC, respectively; P < 0.0001 for all comparisons). For each limbal area, there was no difference between the YC and MAC groups. Results are presented in Figure 3.

In the YC group, the BCE was significantly thicker in the inferior quadrant (47.3 ± 11.9 μm) compared with the nasal (41.1 ± 8.2 μm; P = 0.03), temporal (38.3 ± 7.7 μm; P < 0.0001), and superior (40.4 ± 8.9 μm; P = 0.009) quadrants.

Table 1. Ocular Surface Epithelium Thickness (μm) in Normal Eyes, in Dry Eyes (KCS Group), and in Eyes Treated Chronically with IOP-Lowering Eyedrops (G group)

<table>
<thead>
<tr>
<th>Ocular Surface Epithelium</th>
<th>Young Controls</th>
<th>Middle-Aged Controls</th>
<th>KCS</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>48.3 ± 2.9</td>
<td>83.0 ± 14.3</td>
<td>49.0 ± 4.1</td>
<td>50.3 ± 5.7</td>
</tr>
<tr>
<td>LE</td>
<td>83.0 ± 14.3</td>
<td>84.3 ± 10.1</td>
<td>77.3 ± 17.2</td>
<td>73.3 ± 10.5</td>
</tr>
<tr>
<td>Nasal</td>
<td>67.9 ± 10.7</td>
<td>67.9 ± 7.5</td>
<td>65.0 ± 14.9</td>
<td>68.0 ± 16.1</td>
</tr>
<tr>
<td>Temporal</td>
<td>69.3 ± 14.5</td>
<td>66.9 ± 11.9</td>
<td>63.6 ± 15.0</td>
<td>62.4 ± 13.6</td>
</tr>
<tr>
<td>Inferior</td>
<td>98.0 ± 23.7</td>
<td>106.8 ± 20.0</td>
<td>86.1 ± 21.6</td>
<td>80.3 ± 20.1</td>
</tr>
<tr>
<td>Superior</td>
<td>95.6 ± 15.0</td>
<td>97.2 ± 11.1</td>
<td>95.9 ± 35.4</td>
<td>83.9 ± 18.4</td>
</tr>
<tr>
<td>BCE</td>
<td>42.0 ± 7.5</td>
<td>42.2 ± 7.9</td>
<td>50.4 ± 11.1</td>
<td>49.5 ± 11.0</td>
</tr>
<tr>
<td>Nasal</td>
<td>41.1 ± 8.2</td>
<td>39.1 ± 9.1</td>
<td>46.3 ± 11.0</td>
<td>48.2 ± 12.4</td>
</tr>
<tr>
<td>Temporal</td>
<td>38.3 ± 7.7</td>
<td>41.3 ± 9.8</td>
<td>43.6 ± 11.6</td>
<td>44.6 ± 10.1</td>
</tr>
<tr>
<td>Inferior</td>
<td>47.3 ± 11.9</td>
<td>47.7 ± 15.0</td>
<td>55.2 ± 9.6</td>
<td>53.9 ± 14.3</td>
</tr>
<tr>
<td>Superior</td>
<td>40.4 ± 8.9</td>
<td>43.6 ± 11.7</td>
<td>58.0 ± 27.5</td>
<td>53.2 ± 19.5</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
In the MAC group, there was no significant difference in the epithelial thickness between the four BCE quadrants (nasal, temporal, inferior, and superior: 39.1 ± 9.1 μm, 41.3 ± 9.8 μm, 47.7 ± 15.0 μm, and 43.6 ± 11.7 μm, respectively). For each quadrant, there was no significant difference in BCE thickness between the YC and MAC groups. Results are presented in Figure 4.

Corneal, Limbal, and Conjunctival Epithelia in Ocular Surface Diseases

Between Group Comparisons. Ocular surface epithelium of the KCS and G groups were only compared with the MAC group because these three groups were not different in terms of age (P = 0.237 and P = 0.979, MAC group vs. KCS and G groups, respectively). The mean thicknesses of the CE, LE, and BCE were 49.0 ± 4.1 μm, 77.3 ± 17.2 μm, and 50.4 ± 11.1 μm in the KCS group, respectively, and 50.3 ± 5.7 μm, 73.3 ± 10.5 μm, and 49.5 ± 11.0 μm in the G group, respectively. In both groups, the thickest epithelium was the LE (P < 0.0001 compared with CE and BCE for both groups), but no significant difference was observed between BCE and CE (P = 0.937 in the KCS group and P = 0.670 in the G group). The results are presented in Table 1 and Figure 5.

There was no significant difference in CE thickness between the three groups. The mean LE thickness was significantly lower in the KCS and G groups compared with the MAC group (P = 0.051 compared with KCS and P = 0.001 compared with G) (Fig. 5). The mean BCE was significantly thicker in the KCS and G groups compared with the MAC group (P = 0.005 compared with KCS, and P = 0.017 compared with G) (Fig. 5).

Quadrant Comparisons. As in healthy subjects, the LE thickness was significantly higher in superior (95.9 ± 35.4 μm in KCS and 83.9 ± 18.4 μm in G) and inferior (86.1 ± 21.6 μm in KCS and 80.3 ± 20.1 μm in G) quadrants, compared with nasal (65.0 ± 14.9 μm in KCS and 68.0 ± 16.1 μm in G, P < 0.05 for all comparisons) and temporal (63.6 ± 15.0 μm in KCS and 62.4 ± 13.6 μm in G, P < 0.05 for all comparisons) quadrants. The inferior LE thickness was lower in the KCS and G groups compared with the MAC group (P = 0.0018 and P = 0.001, respectively). In the superior quadrant, the LE was significantly thinner in the G group compared with the MAC group (P = 0.008), but no significant difference was found between the KCS and MAC groups (P = 0.594) (Fig. 6).

In patients with KCS, BCE thickness was significantly higher in the superior (58.0 ± 27.5 μm) compared with the nasal (46.3 ± 11.0 μm, P = 0.020) and temporal (43.6 ± 11.6 μm, P < 0.0001) quadrants, and also significantly higher in the inferior (55.2 ± 9.6 μm) compared with the nasal (P < 0.0001) and temporal (P < 0.0001) quadrants. Also in the KCS group, the BCE was significantly thicker in the superior, inferior, and nasal quadrants compared with values found in the same locations in the MAC group (P = 0.038, P = 0.011, and P = 0.014, respectively) (Fig. 7). In the G group, the BCE thickness was significantly higher in the inferior (53.9 ± 14.3 μm) and superior quadrants (53.2 ± 19.5 μm) compared with the temporal quadrants.
quadrant (44.6 ± 10.1 μm; \( P = 0.006 \) vs. inferior, and \( P = 0.038 \) vs. superior). However, no significant difference was found between the inferior or superior quadrants and the nasal region. The BCE thickness in the nasal quadrant was significantly higher in the G group compared with the MAC group (\( P = 0.010 \)).

**Disease Severity Comparisons.** No correlation was observed between the ocular surface severity grades, the duration of glaucoma treatment, or the IOP, and the LE thickness in the KCS and G groups. In the KCS group, the mean BCE thickness was significantly higher in severity grades 3 and 4 (53.3 ± 12.1 μm) compared with grades 1 and 2 (45.7 ± 7.2 μm) (\( P = 0.031 \)). No significant correlation could be observed between the ocular surface severity grades, glaucoma treatment, or the IOP, and the BCE thickness in the G group.

**DISCUSSION**

The thickness of the three ocular surface epithelia has been previously evaluated in vivo in normal subjects in one study using time-domain OCT. The mean BCE, CE, and LE thicknesses were 44.9 ± 3.4 μm, 54.7 ± 1.9 μm, and 79.6 ± 7.4 μm, respectively. Similarly, using SD-OCT, we observed in normal subjects that the thinnest epithelium was in the BCE (42.0–42.2 μm) followed by the CE (48.3–48.8 μm), and the LE (83.0–84.3 μm). The CE of normal eyes has been evaluated in several studies using OCT, and a higher thickness than we measured has been reported (range: 52 μm to 81 μm). The difference may be attributed to the fact that the other studies used time-domain OCT with an axial resolution of 8.1 to 20 μm, whereas the present study used SD-OCT (Spectralis OCT, Heidelberg Engineering GmbH) with anterior segment module and 3.9 μm axial resolution. A study that used a custom-built SD-OCT to measure CE thickness in normal subjects reported data (52 ± 2.4 μm) similar to our findings. The lower CE thickness observed in the present study may also be explained by the exclusion of the precorneal tear film that measures 7 to 40 μm.

Previous studies evaluated epithelial thickness using the OCT intensity profile where the computer software-controlled cursors were manually placed at the peak of reflectivity corresponding to the tissue interfaces. In the present study, considering the resolution of the OCT images obtained, we directly and manually placed the cursors provided by the SD-OCT software to measure epithelial thickness in each location. Similar to Wang et al.,11 we used the tear film as the first hyperreflective layer and the basal membrane as the second hyperreflective layer. Similar to Tao et al.,18 we found that the group had thinner LE in the inferior and superior quadrants (\( P = 0.001 \) and \( P = 0.008 \) respectively). In the MAC group, the LE was thicker in the inferior quadrant (\( P < 0.0001 \) vs. nasal and temporal) and the superior quadrant (\( P < 0.0001 \) vs. nasal and temporal).
Bowman membrane was located between the second and the third hyperelective layers (i.e., the second and the third peak of reflectivity). Our manual technique has some limitations, such as the placement of cursors precisely at tissue interfaces and obtaining a measurement perpendicular to the ocular surface. However, the computer-assisted image analysis of reflectivity profiles also utilizes the manual placement of a line perpendicular to the ocular surface structures and a manual measurement of the distance between reflectivity peaks. The aim of the present study was to evaluate using a simple method (that could be used in a clinical setting), the cross-sectional thickness of ocular surface epithelia and, despite its limitations, we obtained results similar to studies using a more complex image analysis system.

The CE thickness was not statistically different between the YC and MAC groups. This observation is concordant with the results of previous IVCM studies that showed no alteration in epithelial cell density with age. Similarly, there was no change in limbal or conjunctival thicknesses with age. No other studies have specifically evaluated LE thickness with regard to age, but analyses of the limbus with IVCM have revealed that the presence of Vogt palissades and limbal basal epithelial cells decline with age. However, an age-related cell size enlargement was also observed, which could explain, in part, the stability of the overall epithelial thickness observed using a cross-sectional imaging technique such as OCT. Interestingly, we observed a thicker LE in the superior and inferior regions of each group. These differences are similar to observations reported by Utheim et al. who showed that human limbal epithelial explant of superior origin had high outgrowth success rate and generated epithelia. Also using confocal microscopy and scanning electron microscopy, Shortt et al. showed more limbal crypts in the superior and inferior limbal regions. Wiley et al. used immunofluorescence on corneal sections from donor eyes and found significant regional heterogeneity in the limbus, with a larger number of stem cells in the superior and inferior limbus than in the medial and lateral areas. Thus, the results demonstrate that patients with diseases implicating limbal stem cell deficiency may directly benefit from a noninvasive imaging technique that easily quantifies a parameter (LE thickness) representing or correlating with stem cell density.

The BCE of healthy subjects did not reveal epithelial thickness changes related to age. Similarly, another study using IVCM analyzed conjunctival epithelial cell density and morphology, and did not find any difference between normal subjects of different ages. Another study that used light and electron microscopy on conjunctival biopsies from elderly subjects reported no morphologic changes in BCE thickness, cell arrangement, or goblet cell count in subjects younger than 79 years old.

Two different pathologies that alter ocular surface epithelia were evaluated in this study, namely dry eye and eyes chronically treated for glaucoma or ocular hypertension with IOP-lowering eye drops. There was no significant difference in the CE thicknesses between the KCS, G, and MAC groups. Similar to our observations, IMVC revealed that patients treated with preserved eye drops had a reduced density of corneal superficial epithelial cells and a higher density of basal epithelial cells compared with patients treated with preservative-free eye drops, or the untreated control group. Although the current resolution of SD-OCT may not be sufficient to detect subtle changes in CE thickness, the absence of differences between the KCS, G, and MAC groups could be due to the relative lack of inflammatory cells and immune mediators in the cornea compared with the conjunctiva, rendering the cornea less sensitive to inflammatory changes.

The LE was thinner in the KCS and the G groups compared with the MAC group. To our knowledge, no other in vivo investigation of LE thickness is available in patients with dry eye or in patients treated with IOP-lowering eye drops. These two conditions may induce limbus modifications through chronic inflammation or increased epithelial turnover. The microenvironment of the limbus is considered to be important in maintaining the stemness and renewal of stem cells. Limbal inflammatory conditions may decrease the number of limbal stem cells or alter their functions, resulting in varying degrees of stem cell deficiency. Numerous studies highlight the inflammatory process in dry eye disease. In patients with severe dry eye, a corneal conjunctivalization has been observed using IVCM, suggesting a stem cell deficiency. Similarly, stem cell dysfunction and/or depletion due to eye drop toxicity could also partly explain the LE thinning in the G group. Schwartz and Holland identified the concept of “iatrogenic limbal stem cell deficiency” in eyes treated with antiglaucoma eye drops. Thus, antiglaucoma eye drops and their preservatives, known to be toxic to the corneal and

**FIGURE 7. BCE thickness in each quadrant in KCS, G, and MAC groups. In the KCS group, the BCE was thicker in the inferior (\(P < 0.0001\) vs. nasal and temporal) and the superior (\(P = 0.020\) vs. nasal and \(P < 0.0001\) vs. temporal) quadrants. In the G group, the BCE was thicker in the superior, inferior, and nasal quadrants compared with the MAC group at the same quadrants (\(P = 0.058\), \(P = 0.011\), and \(P = 0.014\), respectively). In the G group, the BCE was thicker in the inferior (\(+P = 0.006\) vs. temporal) and the superior (\(P = 0.038\) vs. temporal) quadrants. In the G group, the nasal BCE was thicker than the nasal BCE in the MAC group (\(P = 0.01\)).

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conjunctival epithelium, could also be toxic to limbal epithelial cells, particularly stem cells. Limbal epithelial thinning as observed with SD-OCT might be the first landmark of these changes.

In contrast to the LE, the BCE thickness was increased in the KCS and G groups compared with the MAC group. Moreover, in dry eye patients, there was a direct relationship between ocular surface disease severity and BCE thickness. In dry eye, this finding is confirmed by ex vivo studies. Kunert et al. demonstrated an increased proliferative activity of the conjunctival epithelium from conjunctival biopsies of eyes with non-Sjögren dry eye. In patients with Sjögren syndrome-related dry eye, Jones et al. observed abnormal proliferation and differentiation at the conjunctival epithelium. Wakamatsu et al. observed with IVCM a decrease in the density of conjunctival epithelial cells in dry eye. However, an increase in epithelial cell size may explain the increased epithelial thickness in dry eye patients. Dry eye condition is associated with conjunctival squamous metaplasia. Several studies using impression cytology or IVCM reported lower goblet cell density and epithelial cell enlargement associated with squamous metaplasia. Interestingly, in the present study, the BCE of patients with dry eye was increased in the superior and inferior quadrants. This could be explained by the increased shear forces under the eyelids on a poorly lubricated ocular surface.

An analysis of conjunctival biopsies revealed a significant increase in BCE thickness and the number of conjunctival epithelial cell layers after topical antiglaucoma treatment. Long-term antiglaucoma topical therapy was found to induce a significant decrease in goblet cells, and an increase in pale cells, macrophages, and lymphocytes within the epithelium. Similar to dry eye, a squamous metaplasia has been reported (via impression cytology) after the use of topical antiglaucoma medications, and might explain our findings. Interestingly, in the G group, the BCE thickness in the nasal quadrant — where the duration of contact between eye drop and ocular surface is prolonged — was significantly higher than in the MAC group. This may result in increased conjunctival irritation and consequently thickening of the BCE.

Despite a lower resolution than IVCM, SD-OCT has numerous advantages over other imaging techniques, such as slit-lamp or ultrasound biomicroscopy, for the evaluation of the ocular surface. OCT is a completely noninvasive imaging method that allows high-resolution analysis and measurements without the need of ocular anesthesia, water bath, or contact procedures. The epithelial thickness measurement of ocular surface tissues seems to be a new promising parameter, useful for the evaluation and grading of ocular surface disease severity. SD-OCT may not only improve our understanding of ocular surface diseases, but could also become a valuable tool in routine clinical practice to evaluate ocular surface epithelia.

References


