Wide Corneal Epithelial Mapping Using an Optical Coherence Tomography

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PURPOSE. To map the corneal epithelium using a map measuring 9 mm in diameter and view the effects of age, sex, and axial length. Additionally, we wanted to demonstrate the reproducibility of this technique.

METHODS. We calculated the epithelial thickness in 220 individuals using an SD-OCT machine with the newly released commercially available algorithm. We included normal eyes with refractive errors between ±5 and −6 diopters (D). We excluded patients with an intraocular pressure of >22 mm Hg, history of cataract, previous ocular surgery, or disease and those with corneal pathology. Additionally, we excluded patients with evidence of systemic disease or pregnancy. Lastly, reproducibility was measured in 50 individuals.

RESULTS. We found the center of the corneal epithelium to be thicker than the peripheral in all zones except the nasal (P < 0.124). The superior quadrant was found to be the thinnest while the inferior was the thickest. Males had a thicker epithelium in all locations except the superior outer section (P = 0.125). Three zones had a weak correlation with age: outer superior (P = 0.059, R = −0.152); outer temporal (P = 0.042, R = −0.150); and outer superior temporal (P = 0.011, R = −0.187). There was no significant relationship with the axial length. We found good to excellent reproducibility when using this technique in the central as well as the peripheral cornea.

CONCLUSIONS. We provide a comprehensive study in healthy, normal eyes using a novel algorithm to map the corneal epithelium with a wide diameter. This study can be used as a reference for future research.

Keywords: normal cornea, corneal epithelium, reproducibility, sex

The first instance of anterior-segment optical coherence tomography (AS-OCT) was described in 1994.1 This was based on a time-domain OCT (TD-OCT) that could generate only corneal pachymetry maps; due to its low resolution of 30 μm, the TD-OCT could not delineate the epithelium. Then, in 2002, the spectral-domain OCT (SD-OCT) was introduced, which drastically increased both the scan speed and resolution.2 This new technique could provide the various layers of the cornea in great detail and therefore automated algorithms were created to generate thickness maps for use in clinical practice.

Previous iterations of the SD-OCT could generate maps that covered only the central 6 mm of the cornea. This limited their applications on peripheral diseases like pellucid marginal degeneration. The newly released software for the SD-OCT device (Avanti RTVue XR; Optovue, Inc., Fremont, CA, USA) has the ability to generate thickness maps that cover a 9-mm corneal diameter, which could potentially increase the diagnostic ability of the machine.

Previously, research has been done on corneal epithelial maps covering 10 mm using a very high frequency (VHF) digital ultrasound.3 However, to our knowledge, no previous study has observed wide epithelial thickness maps with a 9-mm diameter using the SD-OCT. Additionally, we correlated these maps with age, sex, and axial length. Also, keratometric readings were correlated to the central epithelial thickness. Lastly, we evaluated the reproducibility of these automated measurements.

METHODS

Subjects

We conducted a prospective and cross-sectional study at the two centers of Hashmanis Hospital, Karachi, Pakistan. The approval for conducting the study was given by the Ethics Committee of the Hashmanis Hospital in agreement with the tenets of the Declaration of Helsinki. Additionally, we obtained a written informed consent before carrying out any procedures.

This study included patients who reported themselves to be healthy and were between the age of 20 to 75 years. We included one eye for each patient and if both eyes were eligible, we randomly chose one. Ophthalmologic tests included autorefraction (Topcon KR-800, Tokyo, Japan), which contained the keratometry results; best corrected visual acuity (BCVA) using a Snellen chart; intraocular pressure (IOP) using an air-puff tonometer (Reichert 7CR, Reichert, Inc., Depew, NY, USA); dilated fundus examination; slit-lamp examination; axial length measurement (Wavelight OB-820, WaveLight, Erlangen, Germany); and the commercially available SD-OCT device (Optovue, Inc.).

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Submitted: December 23, 2017
Accepted: February 23, 2018

Keywords: normal cornea, corneal epithelium, reproducibility, sex
The patients excluded from the study had a refraction greater than +5 diopters (D) or less than –6 D; BCVA <0.8; IOP >22 mm Hg; any previous ocular surgery; history of cataract; vitreoretinal disease; visual field loss as indicated by clinical examination; glaucoma; ocular hypertension; amblyopia; evidence of systemic disease or pregnancy. Furthermore, patients with corneal diseases, dystrophies, keratoconus and form fruste keratoconus were excluded as well; all patients were screened using an anterior eye segment tomography device (Pentacam HR; Oculus, Wetzlar, Germany). Lastly, patients with dry eyes with a Schirmer’s test 2 value of less than 5 mm were excluded. Additionally, any scan that proved to be of low quality or with evidence of signal blockage was excluded from the study. For each eye included, the pupil was first dilated by administering 1% tropicamide to observe the fundus and do a posterior segment OCT. The eye was then allowed to return to its nondilated state and scanned by an experienced OCT operator.

Reproducibility

We evaluated for the interobserver reproducibility by employing an identical scan protocol in the same patient by two different OCT operators. This was done in 50 patients; 25 of these were male and 25 were female. The mean age group in different OCT operators. This was done in 50 patients; 25 of

Table 2. Epithelial Thickness by Section

<table>
<thead>
<tr>
<th>Section</th>
<th>Inner</th>
<th>Middle</th>
<th>Outer</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>5.8 ± 3.7</td>
<td>5.9 ± 3.5</td>
<td>6.1 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior</td>
<td>5.9 ± 3.5</td>
<td>6.4 ± 3.5</td>
<td>6.8 ± 4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior nasal</td>
<td>5.8 ± 3.6</td>
<td>6.0 ± 3.8</td>
<td>6.2 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nasal</td>
<td>5.2 ± 3.5</td>
<td>5.2 ± 3.3</td>
<td>5.2 ± 3.2</td>
<td>0.124</td>
</tr>
<tr>
<td>Inferior nasal</td>
<td>5.2 ± 3.7</td>
<td>5.2 ± 3.4</td>
<td>5.3 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior</td>
<td>5.0 ± 3.8</td>
<td>5.2 ± 3.8</td>
<td>5.2 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior temporal</td>
<td>5.2 ± 3.7</td>
<td>5.3 ± 3.4</td>
<td>5.2 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temporal</td>
<td>5.3 ± 3.3</td>
<td>5.1 ± 3.0</td>
<td>5.0 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior temporal</td>
<td>5.2 ± 3.5</td>
<td>5.0 ± 3.3</td>
<td>4.7 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values in bold represent statistical significance.

Statistical Analysis

We collected data on Google forms and imported this into the statistical software (SPSS v25; SPSS, Inc., Chicago, IL, USA). This software was used for all further analysis. We used descriptive statistics to calculate mean and standard deviations. A 1-way ANOVA test was used to look for differences between the center and periphery. To correlate age, keratometry, and axial length with corneal epithelial thickness, a Pearson product moment correlation coefficient was used. A partial correlation was used to measure an adjusted P value. Differences among sexes were calculated using the independent t-test. To measure reproducibility we used two tests: the coefficient of variation (CV) and the intraclass correlation coefficient (ICC). Lastly, a multiple regression analysis was used. A value of P < 0.05 was considered to be statistically significant.

RESULTS

Patients

We scanned a total of 331 patients, and included 220 individuals; of these, 107 were males (48.7%) and 113 females (51.3%). The study population had a mean age of 40.0 years with a range of 20 to 75 years. The general characteristics of the patients divided into five age groups are displayed in Table 1.

Excluded Patients

A total of 111 patients were excluded from the study after scanning. The following were the reasons for exclusion: signal blockage (n = 72; 64.9%); evidence of glaucoma (n = 14; 12.6%); central serous chorioretinopathy (n = 10, 9.0%); disc edema (n = 5, 4.5%); optic disc changes (n = 3, 2.7%); age-related macular degeneration (n = 2, 1.8%); previous LASIK surgery (n = 1, 0.9%); nystagmus (n = 1, 0.9%); retinitis pigmentosa (n = 1, 0.9%); and diabetic retinopathy (n = 1, 0.9%).

Corneal Epithelial Thickness by Sector

The mean and standard deviations of the epithelial thickness at different corneal locations is summarized in Table 2. The epithelial thickness was found to be thinnest superiorly with mean values of 51.9 ± 3.5, 48.4 ± 3.5, 44.4 ± 4.4 μm in the inner, middle, and outer locations, respectively. The thickest, on the other hand, was thinnest inferiorly, with mean values 54.0 ± 3.8, 52.9 ± 3.8, and 52.0 ± 4.0 μm in the inner, middle, and outer locations, respectively. Additionally, values toward the center were uniformly greater than those more peripherally, except in the nasal quadrant (P = 0.124).
Variations in normal epithelial thickness profiles can be observed in Figure 1 for males, and Figure 2 for females. While most profiles follow the normal pattern of a thin superior meridian with a thick inferior, and a generally thicker central epithelium, there were a few exceptions present. Some eyes show a thicker peripheral epithelium (Fig. 1, example 1; and Fig. 2, example 5) or a uniform horizontal thickness (Fig. 2, example 6). There were also slight variations in the thickest and thinnest points.
TABLE 3. Differences in Epithelial Thickness by Sex

<table>
<thead>
<tr>
<th>Section</th>
<th>Male,  n = 107</th>
<th>Female,  n = 113</th>
<th>P Value</th>
<th>MR* P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>54.9 ± 3.7</td>
<td>53.0 ± 3.5</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Superior</td>
<td>52.7 ± 3.4</td>
<td>51.1 ± 3.5</td>
<td>0.003</td>
<td>0.010</td>
</tr>
<tr>
<td>Inner</td>
<td>49.2 ± 3.5</td>
<td>47.6 ± 3.5</td>
<td>0.002</td>
<td>0.006</td>
</tr>
<tr>
<td>Middle</td>
<td>44.9 ± 4.2</td>
<td>43.9 ± 4.6</td>
<td>0.123</td>
<td>0.208</td>
</tr>
<tr>
<td>Outer</td>
<td>53.4 ± 3.3</td>
<td>51.9 ± 3.6</td>
<td>0.005</td>
<td>0.026</td>
</tr>
<tr>
<td>Middle nasal</td>
<td>51.5 ± 3.3</td>
<td>50.0 ± 3.2</td>
<td>0.002</td>
<td>0.010</td>
</tr>
<tr>
<td>Outer nasal</td>
<td>49.1 ± 3.5</td>
<td>47.3 ± 4.2</td>
<td>0.003</td>
<td>0.019</td>
</tr>
<tr>
<td>Nasal</td>
<td>54.0 ± 3.6</td>
<td>52.5 ± 3.2</td>
<td>0.003</td>
<td>0.025</td>
</tr>
<tr>
<td>Middle</td>
<td>53.3 ± 3.5</td>
<td>51.8 ± 2.9</td>
<td>0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>Outer nasal</td>
<td>53.5 ± 3.0</td>
<td>52.0 ± 3.3</td>
<td>0.002</td>
<td>0.012</td>
</tr>
<tr>
<td>Inner nasal</td>
<td>54.8 ± 3.7</td>
<td>52.8 ± 3.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Middle nasal</td>
<td>53.8 ± 3.4</td>
<td>51.6 ± 3.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outer nasal</td>
<td>53.3 ± 3.4</td>
<td>51.0 ± 3.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior</td>
<td>55.0 ± 3.6</td>
<td>53.1 ± 3.8</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Middle</td>
<td>54.2 ± 3.7</td>
<td>51.8 ± 3.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outer</td>
<td>53.5 ± 3.6</td>
<td>50.6 ± 3.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior temporal</td>
<td>54.5 ± 3.4</td>
<td>52.8 ± 3.8</td>
<td>0.003</td>
<td>0.016</td>
</tr>
<tr>
<td>Middle temporal</td>
<td>54.0 ± 3.1</td>
<td>52.1 ± 3.5</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Outer temporal</td>
<td>53.6 ± 2.6</td>
<td>51.4 ± 3.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temporal</td>
<td>53.9 ± 3.1</td>
<td>52.3 ± 3.4</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Middle</td>
<td>52.8 ± 2.7</td>
<td>51.1 ± 3.0</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Outer</td>
<td>51.6 ± 2.7</td>
<td>49.9 ± 2.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior temporal</td>
<td>55.2 ± 3.1</td>
<td>51.4 ± 3.6</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Middle</td>
<td>51.0 ± 3.0</td>
<td>49.3 ± 3.4</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Outer</td>
<td>47.9 ± 3.2</td>
<td>46.4 ± 3.3</td>
<td>0.002</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Values in bold represent statistical significance.
* Multiple regression including sex, intraocular pressure, and axial length.

Keratometry

Our mean K1 and K2 values were 43.4 ± 1.5 D and 44.2 ± 1.6 D, respectively. When correlating these values with the central epithelial thickness, we found no correlation for K1 (R = -0.008, P = 0.905) or K2 (R = 0.052, P = 0.653).

Sex

There was no statistically significant difference between the two sexes by age (P = 0.328; IOP (P = 0.334); axial length (P = 0.435); or refractive errors (P = 0.775).

Table 3 reveals the relationship between epithelial thickness and sex. Epithelial thickness was found to be thicker in males at all locations except the superior outer section (P = 0.123). Similar results were obtained when accounting for the sex, axial length, and IOP.

Age and Axial Length

The effects of age on epithelial thickness are displayed in Table 4. A weak negative correlation was seen in the outer superior section (P = 0.039, R = -0.152); outer temporal (P = 0.042, R = -0.150); and outer superior temporal (P = 0.011, R = -0.187). Other epithelial locations and their sections were statistically insignificant.

Table 5 shows the effect of axial length on epithelial thickness; no statistically significant relation was seen between the two mentioned variables.

Reproducibility

The CVs for the inner, middle, and outer circles ranged from 0.023 to 0.031, 0.020 to 0.029 and 0.017 to 0.045, respectively. The ICC ranged from 0.703 to 0.812, 0.712 to 0.917, and 0.796 to 0.930, respectively. The CV and ICC for the center of the cornea was 0.029 and 0.723, respectively. This information is represented in Table 6.

DISCUSSION

Documenting the effects of demographic variables can be vital in catching confounding factors. We must know how the normal epithelium functions in order to draw conclusions about diseased eyes. There are a limited number of studies that have tried to elucidate such effects. Therefore, we have attempted to establish the effects of age, sex, and axial length on normal healthy eyes and elucidate the repeatability of these measurements. This will be useful as a reference article for future studies.
There are several theories for vertical asymmetry in epithelial thickness. First, as hypothesized by Reinstein et al., friction due to blinking abrades the epithelium of the cornea with a larger force applied on the superior meridian. Second, as suggested by Du et al., the constant force applied by the upper eyelid on the superior meridian causes long-term thinning. The upper eyelid covers a greater part of the eye when compared to the lower eyelid and also applies a greater force on the cornea due to gravity. Lastly, as theorized by King-Smith et al., the flow of the tear film with subsequent pooling in the inferior meridian may cause a falsely thick reading.

The last theory can be somewhat negated by the Reinstein et al. study using the VHF digital ultrasound. This is a contact technique that immerses the eye into normal saline. Due to this immersion, the ultrasound has the advantage of excluding the tear film from the analysis. The study concluded that the inferior meridian was thicker than the superior, like our paper. Therefore, it is possible that the OCT may overestimate the inferior meridian due to the tear film; however, this effect is not solely due to this phenomenon.

We propose that the superior meridian is thinner due to the contact time of the tear film being shorter at this location than that of the inferior. Due to this, the lubrication and nourishing effects are less pronounced at the superior meridian causing a faster desquamation of the epithelium and subsequent thinning over time. A previous study has shown a faster transit time of the tear film at the superior quadrants and subsequent pooling in the inferior either due to gravitational effects or structural differences.

**Age**

There is discrepancy in the literature regarding the relationship of age and corneal epithelial thickness as well. Wang et al. and Reinstein et al. found no relationship; the first used a Fourier domain OCT machine and the latter a VHF digital ultrasound. Wu et al., who focused on young myopic patients aged 18 to 40 years using the SD-OCT found a negative correlation. It was proposed by Wu et al. that the younger age group in their study influenced the results. Additionally, a study by Kim et al. found two zones of the corneal epithelium to be inversely related to age: the paracentral and the midperipheral zones. Similarly, another study found the epithelial thickness to thin only in the nasal and limbal zones. Our study found no correlation of age with epithelial thickness in almost all zones and therefore we agree with Wang et al. and Reinstein et al.
This large discrepancy can be explained by the positive correlation of epithelial thickness variability with age; Kanellopoulos et al. described this relationship. They recorded the thickness of the epithelium on four different occasions in 375 individuals and observed the difference in each recording. They found that as a person ages, the reading becomes more variable and hence less reliable. This finding can explain the large discrepancy in modern studies and helps us understand the limitation of using such a technique.

Sex and Axial Length

In this study, males had a thicker corneal epithelium in almost all sections; they had a 1.9-μm thicker epithelium in the center. Kanellopoulos et al. and Wu et al. agreed with our findings; however, their studies show slightly smaller differences in the central epithelium with 1.52 μm and 1.34 μm, respectively. Research has shown the influence of gonadal hormones on ocular development, perhaps this can explain the difference.

One other study has looked at the relationship of epithelial thickness and axial length. Like our study, Wang et al. found no association between the two variables.

Reproducibility

Numerous previous studies have shown good reproducibility of epithelial thickness mapping using SD-OCT. Our study agrees with their findings showing moderate to excellent reproducibility. Additionally, the standard deviations found in this study are similar to those found previously. However, no previous paper has examined the reproducibility of this technique in the periphery of the cornea.

In a preceding paper, Reinstein et al. demonstrated that the OCT suffer signal degradation and a loss of reproducibility as measurements moved peripherally. They theorized that as measurements moved away from the center, the OCT beam did not hit the cornea at the right angles. Due to this, the reflection from the cornea was lost and a sufficient signal was not produced. We demonstrate in this study that advancements in the OCT algorithm have made peripheral measurements of the cornea as reliable as the ones found in the center, which improves the usable scan area.

Advantages

There are several advantages of having an epithelial mapping tool. First, we can measure the epithelial changes after corneal refractive surgery to better understand how procedures like LASIK affect the cornea. Wide mapping will be especially useful for procedures like topography-guided LASIK that use a 9.0-mm ablation zone. Furthermore, this technique can help calculate the minimum thickness for thin flap LASIK. Second, epithelial thickness can help improve the diagnosis of keratoconus. It has been discovered that a pattern of thinning over the tip of the cone with thickening in the surrounding can help diagnose this disease early in its course. This phenomenon was given the name “the doughnut pattern.” Lastly, a wide map can help improve the diagnostic accuracy in diseases of the peripheral cornea, such as the pellucid marginal degeneration.

Limitations

There are several limitations to consider in this study. First, the patients were asked to voluntarily open their eyelids during the exam and there could be differences in the corneal exposure time to the environment. This could induce submic errors in measurement due to variation in the evaporation of the tear film. Second, the cornea has a white-to-white diameter of approximately 11.5 mm and therefore some of the periphery is still missed with this new technique.

Acknowledgments

The authors thank Sameera Irfan for her views on epithelial anatomy, Asif Murad for helping us with the OCT machine and data collection, and Faisal Ahmed, Sameeen Jahangir, and Faraz Haroon for their contribution in data collection. Supported by the Hashmanis Foundation.

Disclosure: N. Hashmani, None; S. Hashmani, None; C.M. Saad, None

References


