The evaluation of the relationship between scleral and conjunctival thickness with age in a healthy Turkish population

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Abstract
Aim: To evaluate the relationship between scleral and conjunctival thickness with age in normal subjects
Material and Methods: One hundred five normal subjects were evaluated in this study. Scleral thickness (ST) and conjunctival thickness (CT) were measured by Spectralis Anterior Segment Module Optical Coherence Tomography (AS-OCT). ST and CT were measured at a distance of 1,000, 2,000, 3,000, 4,000 and 5,000 microns from temporal scleral spur. The mean sclera (STmean) and conjunctival (CTmean) thickness were obtained by the mean of the sclera and conjunctival thickness at 5 points. The axial length (AL) was evaluated by using AL-Scan Optical Biometer (Nidek co.,ltd.). SPSS 21 was used for statistical analysis.
Results: There was no significant difference between males and females according to ST, CT and AL values (p>0.05). There was a significant positive correlation between age and ST values at 2000, 3000, 4000, and 5000 micron apart from temporal scleral spur (r=0.237 p=0.015, r=0.241 p=0.013, r=0.276 p=0.004, r=0.202 p=0.039 respectively). There was a significant negative correlation between age and CT values at 3000, 4000 and, 5000 micron apart from temporal scleral spur(r= -0.222 p=0.023, r= -0.288 p=0.003,r= -0.350 p<0.001). There was a significant positive correlation between age and STmean and, a negative between age and CTmean (r=0.289 p=0.003, r= -0.251 p=0.010).
Conclusion: According to this study ST positively and CT negatively correlated with age significantly.

Keywords: Age; conjunctival thickness; scleral thickness

INTRODUCTION
The sclera is the main structure made of collagen which is responsible for the stable shape of the eye. The sclera is a tissue targeted by emmetropization and myopization. Also, scleral properties are important in glaucoma development and treatment (1). The structure of the sclera may be a cornerstone of some types of glaucoma. Sclera can be affected by many diseases. For example, rheumatologic diseases may affect the thickness of the sclera due to inflammation (2). In some studies, it was reported that scleral thickness varies between genders (3,4). In recent articles, ST was found to vary according to age and ethnicity (5,6).

The sclera layer of the eye is covered by the conjunctiva, a translucent mucus barrier consisting of epithelial and stromal layers of connective and lymphoid tissue (7). The conjunctiva tissue has important roles in mechanical and immune defense (8). Conjunctival thickness was evaluated in some recent studies. Age and diurnal variation may affect the thickness of the conjunctiva (9,10).

ST was evaluated in vitro measurements in a Chinese population (1). Another study evaluated the ST with in vivo measurements (3,4,5,10). Different sides of the sclera had a different radius. ST from different sides was evaluated by some recent studies (11). Also, axial length may affect ST and CT, for this reason, this effect was explored in different studies (1,11)

In previous studies, the scleral thickness was measured by methods such as magnetic resonance imaging, and ultrasound biomicroscopy. However, these methods have handicaps such as image resolution and contact with the eye (11). On the other hand, ST and CT have previously determined using anterior segment optical coherence tomography (3,9). Anterior segment optical coherence tomography (AS-OCT) is a very popular, non-invasive, and easy method for examining the anterior segment of the eye (11). This study aimed to determine ST, CT, and AL in healthy subjects using AS-OCT and AL-Scan Optical Biometer.
MATERIAL and METHOD

This is a prospective randomized study. 105 eyes of 105 patients (61 females, 44 males) were included in the study. Healthy subjects who came to Pamukkale University ophthalmology clinic for normal eye control were included in this study. Ethical approval was obtained from the Ethics Committee (date: 24.10.19, number: 18) and the study was performed according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all patients.

The exclusion criteria included the history of any surgical procedure, trauma, inflammation, refractive errors over ±2 dioptres, glaucoma, and any systemic disease that may affect the sclera and conjunctiva, wearing contact lens, dry eye, using eye drops. All measurements were taken by an expert and repeated until the best image quality was achieved. The image quality was checked whether suitable for scleral and conjunctival thickness measurement. Inadequate images or non-cooperative patients were excluded from the study. To avoid diurnal variations, all measurements in the study were conducted between 9 am and 11 am. All images were evaluated by two blind specialist doctors (UY, HSA).

The axial length (AL) of the eyes was examined using the AL-Scan Optical Biometer (Nidek co.,ltd.). The scleral and conjunctival thicknesses were measured using the AS-OCT. The patient was instructed to look at the nasal side to measure the temporal part of the eye. Scleral thickness was measured at a distance of 1,000, 2,000, 3,000, 4,000, and 5,000 microns from temporal scleral spur. The anterior limit of the sclera was identified by the deep episcleral vascular plexus. It can be seen as a thin hyporeflective space above the sclera tissue (Figure 1). The conjunctival thickness was measured at a distance of 1,000, 2,000, 3,000, 4,000, and 5,000 microns from temporal scleral spur. The conjunctival thickness as defined as the axial distance from the external conjunctival boundary to the anterior scleral boundary (Figure 1). The mean sclera (STmean) and conjunctival (CTmean) thickness were obtained by the mean of the sclera and conjunctival thickness at 5 points.

Scleral thickness was measured at a distance of 1,000, 2,000, 3,000, 4,000 and 5,000 microns from temporal scleral spur (yellow bars). The anterior limit of the sclera was identified by the deep episcleral vascular plexus. The conjunctival thickness was measured at a distance of 1,000, 2,000, 3,000, 4,000 and 5,000 microns from temporal scleral spur. The conjunctival thickness as defined as the axial distance from the external conjunctival boundary to the anterior scleral boundary (red bars).

Figure 1. Scleral and conjunctival thickness measurement

Statistical Analysis

The Statistical Package of the Social Sciences 21.0 software was used for statistical analyses (SPSS Inc., Chicago, IL). Continuous variables were expressed as the mean ± standard deviation, median (minimum and maximum values), and categorical variables as number and percentage. The normal distribution of the data was examined by the Shapiro Wilk test. When the parametric test assumptions were provided, the one-sample t-test was used to compare measurement differences. Mann-Whitney U test was used to compare independent group differences when parametric test assumptions were not provided. Spearman correlation test was used to evaluate the correlation between measurements. P < 0.05 was considered statistically significant.

<table>
<thead>
<tr>
<th>Table 1. The scleral and conjunctival thickness (μ) at different points apart from scleral spur</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST(μ)</td>
</tr>
<tr>
<td>1.000 μ</td>
</tr>
<tr>
<td>2.000 μ</td>
</tr>
<tr>
<td>3.000 μ</td>
</tr>
<tr>
<td>4.000 μ</td>
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<tr>
<td>5.000 μ</td>
</tr>
</tbody>
</table>

ST: Scleral Thickness, CT: Conjunctival Thickness, μ=Micron
The mean age of the subjects was 31.29±13.43. There was no significant difference between sexes according to AL, ST and CT values (p>0.05). The mean AL of the subjects was 23.88±0.63 mm. There was no significant correlation between AL and age, ST, CT values (p>0.05). The ST and CT values measured from 5 different points were significantly different from each other (p<0.001). The ST and CT measurements are seen in Table 1.

The correlation between age and ST measurements are seen in Table 2.
The correlation between age and CT measurements are seen in Table 3.
There was a significant correlation between age and mean ST and mean CT values(r=0.289 p=0.003, r=-0.251 p=0.010).

### Table 2. The correlation between age and ST measurements

<table>
<thead>
<tr>
<th>Age</th>
<th>ST1000</th>
<th>ST2000</th>
<th>ST3000</th>
<th>ST4000</th>
<th>ST5000</th>
<th>STmean</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.064</td>
<td>0.237</td>
<td>0.241</td>
<td>0.276</td>
<td>0.202</td>
<td>0.289</td>
</tr>
<tr>
<td>P</td>
<td>0.519</td>
<td>0.015'</td>
<td>0.013'</td>
<td>0.004'</td>
<td>0.039'</td>
<td>0.003'</td>
</tr>
</tbody>
</table>

r=correlation coefficient  *=p<0.05
ST1000: Scleral thickness at 1000 micron apart from scleral spur, ST2000: Scleral thickness at 2000 micron apart from scleral spur,
ST3000: Scleral thickness at 3000 micron apart from scleral spur, ST4000: Scleral thickness at 4000 micron apart from scleral spur,
ST5000: Scleral thickness at 5000 micron apart from scleral spur. ST mean: The mean of the scleral thickness at 5 points

### Table 3. The correlation between age and CT measurements

<table>
<thead>
<tr>
<th>Age</th>
<th>CT1000</th>
<th>CT2000</th>
<th>CT3000</th>
<th>CT4000</th>
<th>CT5000</th>
<th>CTmean</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.098</td>
<td>-0.149</td>
<td>-0.222</td>
<td>-0.288</td>
<td>-0.350</td>
<td>-0.251</td>
</tr>
<tr>
<td>P</td>
<td>0.322</td>
<td>0.129</td>
<td>0.023'</td>
<td>0.003'</td>
<td>0.0001'</td>
<td>0.010'</td>
</tr>
</tbody>
</table>

r=correlation coefficient  *=p<0.05
CT1000: Conjunctival thickness at 1000 micron apart from scleral spur, CT2000: Conjunctival thickness at 2000 micron apart from scleral spur,
CT3000: Conjunctival thickness at 3000 micron apart from scleral spur, CT4000: Conjunctival thickness at 4000 micron apart from scleral spur,
CT5000: Conjunctival thickness at 5000 micron apart from scleral spur, CTmean: The mean of conjunctival thickness at 5 points

**DISCUSSION**

In this study, it was found that the ST increased with age, and CT decrease with age. The axial length did not change according to age. Sclera and conjunctiva were measured in previous studies. It is known that the scleral thickness may vary according to race in previous publications (6,12). This is the first scleral and conjunctival thickness measurement study in a healthy Turkish population.

In a study of 281 globes in China, it was shown that scleral thickness was not affected by age in individuals over 2 years of age (1). However, another study showed that scleral thickness increases with age in young adults (5). In another study, it was shown that scleral thickness increased with age in adults (11). Some studies have shown that scleral thickness is affected by sex and is thicker in males (5,3). In our study, we found that ST increases with age. Also, there was no significant difference between males and females.

Since sclera has a different radius in different regions, scleral thickness will have different values in different regions. In the study of Ebneter et al., the scleral thickness was measured 2 mm from the scleral spur in inferonasal, inferotemporal, superotemporal and supranasal quadrants, and the thicknesses in all quadrants were significantly different (11). In the study of Buckhurst et al., the scleral thickness was measured from 8 meridians and a significant difference was found between the meridians (3). In our study, we measure ST and CT only temporal quadrant but there were 5 different points apart from scleral spur. In our study, the values measured from 5 different points were statistically different from each other.

Conjunctival thickness was measured in previous studies (9,13). Read et al. showed that conjunctival thickness was significantly thicker at younger ages. In the same study, it was shown that conjunctival thickness was not affected by refractive errors (5). In another study, it was shown that conjunctival thickness showed diurnal rhythm, and the thickest time was awakened (10). In another study, it was shown that conjunctival thickness showed diurnal rhythm and the thickest time was immediately after awakening (10). In our study, CT correlated negatively with age significantly. Our study showed that CT was significantly thicker at young people. This result is consistent with the studies mentioned above.

The relationship between AL and ST, CT has been investigated in previous studies (3,5). Read et al. showed that axial length was not associated with the sclera and
conjunctival thickness (5). In another study, no correlation was found between axial length, refraction errors, and scleral thickness (3). In our study, no difference was found between AL, sclera, and conjunctival thickness measurements. The fact that AL did not affect these measurements may be since the scleral thickness change in myopia patients is mostly in the posterior part of the globe (14,15).

There were some limitations in our study. First, we did not evaluate the refractive errors of the patients. But in a past study, it was found no correlation between refractive errors and scleral thickness (3). Second; we only measure the temporal side of the eye, which may be the other quadrants of the eye measured and evaluated.

CONCLUSION

According to our results, ST increases with age and CT decreases with age. With the effect of matrix metalloproteinases, collagen structure changes with aging (16). This may affect the thickness of sclera and conjunctiva. Our study will contribute to the literature to know age-related changes in the sclera and conjunctiva.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: Pamukkale University Non Interventional Clinical Research Ethics Committee (date:24.10.19, number:18).

REFERENCES