Scleral Intraocular Pressure Measurement in Cadaver Eyes Pre- and Postkeratoprosthesis Implantation

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**Purpose.** We correlated scleral IOP to assigned IOP using pneumatonometry in cadaver eyes before and after Boston type I keratoprosthesis (KPro) implantation.

**Methods.** Corneal IOP and scleral IOP at the superonasal, superotemporal, inferotemporal, and inferonasal quadrants were measured using pneumatonometry in six cadaver eyes cannulated with an infusion line with assigned IOP held at 20, 30, 40, and 50 mm Hg. Measurements of scleral IOP at the same location were repeated after a KPro was implanted. Correlations between scleral IOP and assigned IOP were analyzed for the entire group of eyes, and for each individual eye before and after KPro. One eye was tested by another masked grader for interobserver variability.

**Results.** Scleral IOP measured higher than corneal IOP by a mean of 13.2 mm Hg. For group analysis, pre-KPro scleral IOP had a positive and linear correlation with assigned IOP in all quadrants (P < 0.00001), and this correlation was preserved after KPro implantation (P < 0.00001). There was strong interobserver agreement in all measurement sites (P < 0.001). In analyses of individual eyes, scleral IOP measured at the inferotemporal quadrant confirmed the strong linear association between scleral IOP and assigned IOP before and after KPro for all study eyes. A Bland-Altman plot showed that the difference in scleral IOP between pre-KPro and post-KPro eyes fell mostly within ±5 mm Hg.

**Conclusions.** Scleral IOP measured by pneumatonometry may be used to estimate IOP in cadaver eyes with and without keratoprosthesis. This may be a potential modality for assessing IOP for patients with corneal pathology or keratoprosthesis.

Keywords: keratoprosthesis, intraocular pressure, scleral pressure, glaucoma anterior segment

Multiple methods of measuring IOP exist, but most of the commonly employed methods, including Goldmann applanation, pneumatonometry, and Tonopen applanation, all require measurement over the cornea. Corneal pathology, including scarring, edema, and thinning, can affect IOPs measured with these approaches, rendering them inaccurate.1–4 In extreme cases, severe corneal opacification may preclude IOP readings.

Similarly, IOP cannot be measured with these traditional approaches in patients who have received a keratoprosthesis. The Boston Type I keratoprosthesis (KPro) has been gaining popularity since its Food and Drug Administration (FDA) approval in 1992, with nearly 1200 implanted in 2010, an exponential increase from less than 100 cases in 2002.5 However, IOP monitoring in KPro patients is challenging. The central cornea is replaced by synthetic material and, therefore, is unavailable for IOP measurements. Currently, digital palpation is the recommended method for monitoring IOP in KPro eyes,6 but the accuracy can be highly dependent on the experience of the grader.7,8

Glaucoma is the most common complication leading to irreversible vision loss in KPro patients, with a prevalence of 85% to 89%.9–11 The reasons for this high rate of glaucoma are multifactorial. The KPro candidates often have significant ocular pathology, including a preoperative diagnosis of glaucoma.9,10 In addition, IOP can increase due to surgically-induced inflammation, pigment dispersion, steroid response, or angle crowding.10 The KPro implants also may change scleral rigidity and the biomechanical forces of the eye, further contributing to optic nerve damage.10 Despite close monitoring and treatment, definite glaucoma progression is documented in 22% of KPro patients.11 End-stage glaucoma is the most common factor leading to vision loss in these patients with vision worse than 20/200.11 These findings beg a better modality to detect subtle changes in IOP to initiate early glaucoma treatment in KPro patients.

Prior studies have attempted to measure IOP at locations other than the central cornea. Khan et al.12 reported fair reliability between limbal and corneal Tonopen IOP measurements in normal cadaver eyes between 10 and 35 mm Hg. However, the proximity of the bulky Boston Type I KPro unit to the limbus and the change in rigidity at the corneal host–donor junction would likely preclude reliable IOP readings. Tonopen IOP measurements at the sclera in normal human eyes have been found to correlate poorly with central corneal IOP.12,13 Pneumatonometry, on the other hand, has been shown to have
the best correlation with manometry in vivo and in vitro when used at the central cornea. In addition, pneumatonometry is useful in severely diseased corneas that are edematous and scarred, where distortion of mires prevents reliable readings with applanation. A recent study showed a linear association between scleral IOP and corneal IOP for healthy subjects. However, it is unknown whether pneumatonometry can provide reliable IOP measurement at the sclera when IOP is increased to pathologic levels or after a KPro is implanted.

This study aimed to analyze the relationship between scleral IOP and true IOP by using pneumatonometry before and after Boston Type I KPro placement to ascertain if scleral IOP can be a reliable alternative to measuring true IOP in KPro eyes or eyes with other corneal pathology.

**METHODS**

Six cadaver eyes were oriented anatomically based on the insertion of the inferior oblique muscle and optic nerve. Discrete marks were placed over the center of the cornea and on the sclera 3 mm posterior to the limbus at the 10:30, 1:30, 4:30, and 7:30 clock hour positions. A 23-gauge infusion cannula was inserted into the anterior chamber through the temporal limbus (Fig. 1A) and connected to an Accurus vitrectomy machine (Alcon, Inc., Fort Worth, TX, USA). Utilizing positive infusion pressure with balanced salt solution (BSS), the eye initially was pressurized to 15 mm Hg to confirm a closed system with watertight wounds. The IOP was confirmed by manometry in one eye. The eye then was pressurized to 20 mm Hg. After the pressure was permitted to equilibrate for 5 minutes, three successive IOP measurements were taken with a pneumatonometer (Mentor Model 30 classic; O&O, Inc., Norwell, MA, USA) over each of the five previously marked sites: central cornea, superonasal sclera, superotemporal sclera, inferotemporal sclera, and inferonasal sclera. Pneumatonometer readings were accepted when the tracing reached a steady oscillatory line for 5 seconds and the standard deviation as measured by the machine was less than 0.2. The same procedures were followed after the eye was pressurized sequentially to 30, 40, and 50 mm Hg. The infusion tubing then was clamped and disconnected from the vitrectomy machine.

A KPro keratoprosthesis with an 8.5 mm back plate was implanted in 5 cadaver eyes using the cadaveric cornea as a carrier. A pediatric size 7.0 mm KPro back plate was used for one cadaver eye. In brief, the cadaveric cornea was trephinated with an 8.5 mm or a 7 mm metal trephine. A 3.0 mm hole was created in the center of the cornea with a dermatologic punch trephine, and the cornea was placed onto the stem of the anterior optic. The back plate then was placed onto the stem and gently secured using the supplied hollow pin. The titanium locking ring was not used to allow for KPro reuse in subsequent cadaver eyes. The corneas were sutured back into the cadaver eyes using 16 interrupted 10-0 nylon sutures (Fig. 1B).

The infusion tubing was reconnected to the Accurus vitrectomy machine (Alcon, Inc.). The aforementioned protocol was repeated to obtain IOP measurements at the same assigned IOP at 20, 30, 40, and 50 mm Hg. The presence of a KPro precluded IOP measurement over the central cornea so only scleral IOP measurements at the four quadrants were taken following KPro placement. Wound integrity was confirmed with Seidel testing at each IOP level. A single unmasked grader (YH) measured IOP for all eyes. To control for observer bias, a masked grader (MCC, see acknowledgments) measured IOP for one eye.

Bivariate regression was performed to analyze the relationship between assigned IOP and measured scleral IOP at each of the chosen sites before and after KPro placement. We compared the relationship between assigned IOP and scleral IOP pre- and post-KPro by fitting a linear mixed effects regression with assigned IOP and KPro as predictors, along with the interaction term, and contrasting this with the model including assigned IOP alone (likelihood ratio χ²). A random slope and intercept were included. The mixed effect regression equation is as follows. Let the eyes be labeled \( i = 1, \ldots, 6 \). For each eye, we have repeated measurements \( j = 1, \ldots, 4 \). Let the true pressure at the \( j \)-th measurement be denoted \( P_j \). Let the
outcome variables (scleral pressure) be $Y_{ij}$. Then the model is described by

$$Y_{ij} = \beta_0 + \beta_1I_{ij} + u_{0i} + u_{1i}P_{ij} + e_{ij},$$

where $\beta_0$ is the intercept, $\beta_1$ the slope, $u_{0i}$ is a random intercept and $u_{1i}$ a random slope; $e_{ij}$ is random error. Comparisons of slopes and intercepts between eyes were conducted using each eye as a fixed effect (ANCOVA). Scleral IOP taken at the same location in the same eye before and after KPro implantation was used to plot the difference of these two measurements against the mean, yielding a Bland-Altman plot. Bland-Altman plots constructed for dependent observations exhibit error estimates determined by resampling eyes. Because of the dependence of observations taken on the same eye, bootstrap resampling of cases was used when assessing statistical significance. Intraclass correlations were calculated to compare results from the unmasked and masked graders. All computations were done in R version 2.10 for Macintosh (R Foundation, Vienna Austria, available in the public domain at http://www.r-project.org). This research adheres to the tenets of the Declaration of Helsinki.

RESULTS

Among the six cadaver eyes, four were right eyes and two were left eyes. The duration from time of death to time of study was between 2 and 5 days, with a mean of 3.5 days. All eyes were pseudophakic. In the group analysis, we found that before KPro implantation, the average IOP measurements from each of the four scleral quadrants (scleral IOP) were higher than the assigned IOP by the following amounts: 10.0 $\pm$ 2.9 mm Hg at the superonasal quadrant, 16.1 $\pm$ 7.8 mm Hg at the inferonasal quadrant, 12.6 $\pm$ 1.6 mm Hg at the superotemporal quadrant, and 14.0 $\pm$ 5.4 mm Hg at the inferotemporal quadrant. In addition, a strong linear relationship existed between the assigned IOP and scleral IOP in all four quadrants: superonasal (slope $= 0.99$, 95% confidence interval [CI], 0.89–1.09; $R^2 = 0.89$; $P < 0.0001$; Fig. 2A), inferonasal (slope $= 0.96$, 95% CI, 0.78–1.14; $R^2 = 0.89$; $P < 0.0005$; Fig. 2B), superotemporal (slope $= 0.84$, 95% CI, 0.73–0.95; $R^2 = 0.92$; $P < 0.0001$; Fig. 2C), and inferotemporal (slope $= 0.99$, 95% CI, 0.89–1.09; $R^2 = 0.88$; $P < 0.0001$; Fig. 2D). Full regression models accompany Figures 2A to 2D. The central corneal IOP was slightly higher than the assigned IOP by 3.78 mm Hg, and a linear relationship also was seen between assigned IOP and central corneal IOP (slope $= 0.94$, $R^2 = 0.99$, $P < 0.0001$, Fig. 3).

A similarly strong linear relationship between the assigned IOP and scleral IOP measurements was observed after KPro placement in all four scleral quadrants: superonasal (slope $= 0.99$, 95% CI, 0.77–1.22; $R^2 = 0.88$; Fig. 2A), inferonasal (slope $= 1.00$, 95% CI, 0.74–1.27; $R^2 = 0.53$; Fig. 2B), superotemporal (slope $= 0.94$, 95% CI, 0.80–1.07; $R^2 = 0.80$; Fig. 2C), and inferotemporal (slope $= 0.96$, 95% CI, 0.88–1.05; $R^2 = 0.70$; Fig. 2D). There was no statistically significant difference between the pre- and post-KPro relationships of assigned IOP and scleral IOP for the superonasal quadrant ($P = 0.54$), inferonasal quadrant ($P = 0.45$), superotemporal quadrant ($P = 0.06$), and inferotemporal quadrant ($P = 0.65$). Insufficient data were available to detect any differences between the 7 mm (one eye) and 8.5 mm keratoprosthesis back plate (5 eyes), but a linear relationship existed.

We further analyzed the relationship between assigned IOP and scleral IOP for each individual eye measured from the inferotemporal quadrant. Consistent with group analysis, for each individual eye, there was a significant linear relationship between assigned IOP and inferotemporal scleral IOP before KPro (Fig. 4, solid lines; Table). The associations between inferotemporal scleral IOP and assigned IOP for each eye were similar, with the slopes of the regression lines nearly parallel to one another ($P = 0.71$, ANCOVA). However, the intercepts of the regression lines differed ($P < 0.0001$, linear model). After KPro implantation, there also was a linear relationship between scleral IOP and assigned IOP for each eye (Fig. 4, dashed lines; Table). There was no evidence that keratoprosthesis implantation significantly changed the association between inferotemporal scleral IOP and assigned IOP ($P = 0.77$, linear mixed model, likelihood ratio test)

A Bland-Altman plot (Fig. 5) showed that the difference between pre-KPro and post-KPro scleral IOP fell between $-5$ and $+5$ mm Hg in 75% of the data points, and between $-2$ and $+2$ mm Hg in 45.8% of the data points, with a range of $-9.5$ to $+8.5$ mm Hg. Moreover, the mean of the differences was 0.39 mm Hg and the standard deviation of the differences was 9.2% of the mean. The plotted data points showed no evidence of a relationship as the mean IOP increases ($P = 0.83$, cases bootstrap), suggesting that the difference in scleral IOP pre- and post-KPro did not depend on the mean scleral IOP.

There was strong interobserver agreement between unmasked and masked IOP measurements at all sites: central cornea (intraclass correlation coefficient [ICC] = 0.99, $P < 0.0001$), superonasal sclera (ICC = 0.97, $P < 0.0001$), inferonasal sclera (ICC = 0.94, $P < 0.0001$), superotemporal sclera (ICC = 0.95, $P < 0.0001$), and inferotemporal sclera (ICC = 0.93, $P < 0.0001$).

DISCUSSION

Consistent with prior studies, this study showed that individual scleral IOP readings are not representative of assigned IOP and generally measure higher than assigned IOP by 10 to 16 mm Hg. In addition, for given assigned IOP, significant variation existed in scleral IOP measurements among eyes. For example, when the assigned IOP was set to 50 mm Hg, scleral IOP measurements ranged from 53 to 72 mm Hg for the six eyes. Thus, a single scleral IOP reading is not reliable or useful in predicting true IOP.

However, in group and individual eye analyses, we found a strong linear relationship between pneumatonometry-measured scleral IOP and assigned IOP in cadaver eyes pre- and postkeratoprosthesis implantation (Figs. 2, 4). This suggested that serial scleral IOP measurements by pneumatonometry can help determine true IOP. Not only can the true IOP in pre-KPro eyes be deduced by using scleral IOP measurements and the regression equation developed before KPro placement, but post-KPro IOP also may be calculated in the same way as this relationship persists after KPro implantation. Group and individual analyses also revealed a linear relationship between scleral IOP and assigned IOP with slopes close to 1 in most quadrants. This indicates that a change in scleral IOP represents a true change in assigned IOP in a 1:1 relationship. For example, an increase of 10 mm Hg in scleral IOP signifies an increase of 10 mm Hg in assigned IOP. Therefore, once a scleral and true IOP for a given eye is obtained at baseline, a subsequent change in scleral IOP measurements suggests a change in true IOP by the same degree. The same scenario can be applied to eyes in which corneal disease precludes reliable corneal IOP measurements. Assuming that the association between scleral IOP and true IOP is similar between the two eyes of the same patient, one can estimate the true IOP in the diseased eye based on the relationship developed from the fellow eye. If these findings are confirmed in vivo, scleral IOP can be extremely helpful in monitoring IOP in KPro patients or patients with corneal pathology.
We measured scleral IOP at four different scleral quadrants and found that all quadrants have regression models fitting a slope close to one except the superotemporal quadrant. Several reasons may explain this aberration. One possibility is that our anterior chamber infusion cannula was located near the superotemporal quadrant, which may affect the adjacent scleral measurements. Second, the scleral property at each quadrant may be intrinsically different. Studies have found that the superotemporal quadrant has the lowest resistance (highest compliance) to force, whereas the inferonasal quadrant has the highest resistance. A third factor that may affect scleral IOP measurements is scleral pathology. Conditions, such as scleromalacia or a scleral plaque, can change scleral rigidity and thickness, and, thus, alter the relationship between the scleral IOP and assigned IOP. Two of the six cadaver eyes in our study had superotemporal scleral thinning with underlying uveal tissue discoloration visible to the naked eye. When data from these two eyes were excluded, the regression slope for the superotemporal quadrant more closely approximates 1, which is similar to the slopes for the underlying collagen infrastructure, which has been shown to have different orientations based on location. A regression model of scleral IOP measured over the superonasal sclera and assigned IOP in pre-KPro (black) and post-KPro (red) cadaver eyes; dotted line represents linear correlation with slope of 1. The regression equations were included for pre-KPro and post-KPro. Regression model of scleral IOP measured over the inferonasal sclera and assigned IOP in pre-KPro (black) and post-KPro (red) cadaver eyes; dotted line represents linear correlation with slope of 1. The regression equations were included for pre-KPro and post-KPro condition. Regression model of scleral IOP measured over the superotemporal sclera and assigned IOP in pre-KPro (black) and post-KPro (red) cadaver eyes; dotted line represents linear correlation with slope of 1. The regression equations were included for pre-KPro and post-KPro condition. Regression model of scleral IOP measured over the inferotemporal sclera and assigned IOP in pre-KPro (black) and post-KPro (red) cadaver eyes; dotted line represents linear correlation with slope of 1. The regression equations were included for pre-KPro and post-KPro condition.
other quadrants. However, this is purely observational, as we cannot draw any definite conclusion due to our small sample size. Studies of LASIK eyes have shown that corneal elasticity and thickness affect central corneal IOP measurements. Likewise, variations in scleral thickness and rigidity may affect scleral IOP measurements.

We performed group and individual analyses in this study. While a group analysis minimizes noise and provides a generalized equation to predict post-KPro IOP, individual eye analyses allow for assessing similarities and differences between the eyes given the possible unique properties of each individual eye. We chose the inferotemporal scleral quadrant for the individual analyses because in a clinical setting, scleral pressure is most likely measured at this location. Intraocular surgeries, such as glaucoma procedures, are performed uncommonly in the inferotemporal quadrant, and this location is most easily accessible without possible obstruction by the patient’s nose or a tight upper lid. The similar results found in the group and individual analyses confirm that the linear relationship between scleral IOP and assigned IOP before KPro implantation is preserved in post-KPro eyes. We found that the slopes between each eye do not differ significantly, but the intercepts of the regression lines do. This indicates that while the linear 1:1 relationship is similar between eyes, the absolute value of scleral IOP varies significantly from corneal IOP among eyes. This finding is not surprising given that previous studies have found a wide range of measured scleral IOP, leading to differences in scleral properties as discussed above. It further emphasizes that the “intercept” for each eye needs to be individually assessed.

Lastly the Bland-Altman plot shows that the difference between pre-KPro and post-KPro scleral IOP falls mostly between ±5 mm Hg, and the data points were in random distribution. This suggests that the difference in scleral IOP pre- and post-KPro does not depend on the mean of the two, which again confirms that KPro implantation does not alter the relationship between scleral IOP and assigned IOP.

Recently, Kapamajian et al. reported a linear correlation between scleral IOP and corneal IOP in normal subjects, albeit with different regression equations. Our study design differs from theirs in that they have one-time scleral IOP readings, while we have a series of scleral IOP measurements associated with a gradient of assigned IOPs for each eye. Because scleral IOP depends on scleral thickness, rigidity, and the presence or absence of pathology, there may be a large variation in scleral IOP. Our study design obviates this inherent variable by

<table>
<thead>
<tr>
<th>Eye</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
<th>P Value for Regression Model†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-KPro Eye #1</td>
<td>1.02 (0.76, 1.28)</td>
<td>21.73 (12.10, 31.37)</td>
<td>0.017</td>
</tr>
<tr>
<td>Eye #2</td>
<td>0.97 (0.71, 1.23)</td>
<td>13.65 (4.01, 23.29)</td>
<td>0.019</td>
</tr>
<tr>
<td>Eye #3</td>
<td>0.97 (0.67, 1.28)</td>
<td>15.85 (4.64, 27.06)</td>
<td>0.025</td>
</tr>
<tr>
<td>Eye #4</td>
<td>0.82 (0.76, 0.88)</td>
<td>6.82 (4.71, 8.92)</td>
<td>0.001</td>
</tr>
<tr>
<td>Eye #5</td>
<td>1.10 (0.93, 1.26)</td>
<td>14.53 (8.43, 20.64)</td>
<td>0.006</td>
</tr>
<tr>
<td>Eye #6</td>
<td>1.04 (0.85, 1.23)</td>
<td>11.17 (4.13, 18.20)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Post-KPro Eye #1 | 0.90 (0.84, 0.96) | 23.70 (21.46, 25.94) | 0.001 |
| Eye #2 | 0.97 (0.78, 1.16) | 11.75 (4.70, 18.80) | 0.010 |
| Eye #3 | 1.06 (0.85, 1.27) | 18.88 (11.10, 26.67) | 0.010 |
| Eye #4 | 0.89 (0.74, 1.04) | 4.17 (−1.45, 9.76) | 0.007 |
| Eye #5 | 0.89 (0.57, 1.21) | 17.55 (5.78, 29.32) | 0.032 |
| Eye #6 | 1.08 (0.88, 1.28) | 9.93 (2.55, 17.32) | 0.009 |

P value‡ | 0.71 | <0.0001 |

The regression equation for each eye is: scleral IOP = slope × assigned IOP + intercept. Linear mixed effects regression with assigned IOP and KPro as predictors (likelihood ratio χ²). Comparisons of slopes and intercepts between eyes were conducted using each eye as a fixed effect (ANCOVA).

The table above provides the regression analyses data for measured inferotemporal scleral IOP versus assigned IOP for all six cadaver eyes pre-KPro and post-KPro.
measuring the change in scleral IOP for each eye, using itself as baseline reference rather than another eye. Therefore, the correlation of inferotemporal scleral IOP and corneal IOP in our study ($r = 0.94$) is much stronger than theirs ($r = 0.57$). In addition, Kapamajian et al. tested healthy eyes with an IOP range of 10.5 to 27 mm Hg. It is not known whether the correlation they reported applies to eyes with IOP outside of this range. In contrast, our regression equation was calculated from an IOP gradient up to 50 mm Hg to mimic pathologic conditions.

There are a few limitations to this study. First, we used a small number of cadaver eyes. In addition, the conjunctiva had been removed previously during processing, an anatomic change that could affect pneumatonometer readings. Furthermore, the effect of scleral rigidity and thickness on scleral IOP measurements must be elucidated in future studies. For example, the size of the KPro back plate may contribute significantly to scleral rigidity. In our study, most eyes received the 8.5 mm back plate except one eye that received the 7 mm back plate. We did not find a significant difference between the two sizes, but we have insufficient data to draw any definitive conclusions. In vivo studies are needed to verify our findings in human eyes where conjunctiva, scleral rigidity, extraocular muscles, and direction of gaze may come into play. Ideally, cadaveric eyes should be as fresh as possible, but the mean death to study time for the eyes used here was 3.5 days. Also, while manometry was employed to confirm the IOP of the system, independent simultaneous IOP measurements in addition to pneumatonometry were not obtained. Finally, although interobserver correlation for data collected between masked and unmasked graders was extremely high, masking all graders would remove this potential source of bias.

Despite these limitations, results from this study suggested that measuring scleral IOP with pneumotonometry may offer an alternative to digital palpation for monitoring glaucoma in eyes in which conventional IOP measurements cannot be employed, such as those that have undergone KPro placement. Efforts to develop a radiofrequency-based intraocular IOP transducer are underway, and will, in principle, provide very accurate IOP measurements. However, this technology still is under development and may be invasive. For now, we are cautiously optimistic that measuring scleral IOP can provide a more immediate solution utilizing currently available technology.

In summary, this study showed that there is a linear relationship between scleral IOP and assigned IOP across a wide range of IOP in cadaver eyes, and that this correlation is highly preserved after Boston KPro implantation. Indeed, measuring baseline scleral IOP before KPro implantation may provide the clinician important information for following true IOP after KPro implantation.

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