Influence of Ocular Stiffness on Intraocular Pressure Estimation Using Goldmann Applanation Tonometry

Masako Shirane[1,*] Yoshiaki Kiuchi[1] Keiko Otani[2] Yuichi Kurita[3]

Makoto Kaneko[4] Joji Takenaka[1]

[1]*Department of Ophthalmology and Visual Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8553, Japan*
[2]*Department of Environmetrics and Biometrics, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan*
[3]*Institute of Engineering, Hiroshima University, Hiroshima 739-8527, Japan*
[4]*Department of Mechanical Engineering, Osaka University, Osaka 565-0871, Japan*

Received 29 Nov 2012; Accepted 1 Feb 2013; doi: 10.5405/jmbe.1381

Abstract

Accurate intraocular pressure (IOP) measurement is important for management of glaucoma. Ocular stiffness is considered to be an important biomechanical factor influencing corneal deformation during IOP measurements by a Goldmann applanation tonometer (GAT). The purpose of this study was to investigate how ocular stiffness is related to clinical parameters. Ocular stiffness was defined as the ratio between the change in the force applied to the cornea and the resultant displacement of the corneal apex. Fifty-nine Japanese subjects had their ocular stiffness measured. A GAT was used to apply pressure to the cornea and a high-resolution camera was used to measure corneal apex displacement by photographing the cornea in profile. Multiple regression analyses were used to assess how ocular stiffness is associated with seven factors: IOP measured by the GAT (IOP_{GAT}), radius of curvature of the cornea (R), ocular axial length, anterior chamber depth, central corneal thickness, age, and gender. Ocular stiffness was found to be proportional to the product of $(IOP_{GAT})^{0.66}$ and $(R)^{0.92}$; the other factors were not significantly associated with ocular stiffness. For an individual eye, because the value of the radius of corneal curvature was unchanged, ocular stiffness was proportional to $(IOP_{GAT})^{0.66}$. This means that the rate at which ocular stiffness diminishes is greater in the lower, compared to the higher, IOP_{GAT} range. Hence, the rate at which the true IOP diminishes is greater in the lower IOP_{GAT} range because the true IOP is considered to be the primary factor influencing ocular stiffness. This suggests that, in patients with lower IOP_{GAT}, the change of true IOP should become progressively greater than the value indicated by IOP_{GAT}. This result allows improved evaluation of treatment effectiveness in normal-tension glaucoma patients.

Keywords: Ocular stiffness, Intraocular pressure estimation, Goldmann applanation tonometry, High-resolution camera, Glaucoma

1. Introduction

Glaucoma is one of the leading causes of irreversible blindness. Reduction of intraocular pressure (IOP) is the only effective, evidence-based treatment. Ascertaining effectiveness of therapy necessarily requires clinicians to evaluate a patient's IOP, but accurate estimation of IOP is an ongoing clinical challenge. IOP can be measured using various instruments. Goldmann applanation tonometer (GAT) was invented by Goldmann [1], and has since been the most widely used instrument for IOP measurement, owing to its ease of use, accuracy, and reproducibility of measurement. Non-contact tonometers [2], among other instruments, were later developed, but their degree of reliability has not matched that of the GAT (i.e., the measured IOPs vary). Therefore, the GAT has remained the standard choice for IOP measurement.

IOP measured by the GAT (IOP_{GAT}) is based on the force needed to achieve the applanation of a circular zone with a diameter of 3.06 mm on a cornea. This method assumes that the eye is an ideal globe that follows Imbert-Fick's law, i.e., the eye is filled with liquid and has an infinitely thin and homogeneous shell with no elasticity. In reality, however, the eye has a heterogeneous shell, which consists of the cornea and sclera backed by the retina and uvea, and the shell has thickness and bending flexibility. The contents of the eye are also heterogeneous and change with age. Therefore, IOP_{GAT} must be evaluated with a deeper understanding of the structural

* Corresponding author: Masako Shirane
Tel: +81-82-5813383; Fax: +81-82-5810187
E-mail: shirane-eye@hiroshima.email.ne.jp

complexity of the eye and other factors (e.g., ocular biomechanical properties) influencing corneal deformation during IOP measurement.

Many studies have investigated corneal parameters that influence IOP measurement. Some of those studies have shown that thin corneas are associated with an underestimation of IOP [2-7], more notably when non-contact tonometers are used [2,8]. There have also been studies on the relationship between IOP and other ocular parameters. For example, Tomlinson and Phillips [9] reported a significant positive correlation between axial length and IOP, and suggested that volume of the globe most likely influences measured IOP. A positive correlation between axial length and IOP was also found by Pärssinen [10].

There has recently been increased interest in the relationship between IOP and the biomechanical properties of the cornea and eyeball. Some studies have shown that higher IOPs are correlated with higher corneal elasticity [11-13]. Kiuchi *et al*. [14] reported that lower IOP significantly affects the degree of corneal displacement during tonometry with a non-contact tonometer. Another study reported that rigidity of the eye was significantly greater in eyes with hypertensive primary open angle glaucoma (POAG) compared to that of normal eyes [15]. However, the number of studies exploring how ocular biomechanical properties affect GAT-based IOP measurements is limited.

The present study considers ocular stiffness to be an important biomechanical factor influencing corneal deformation during IOP measurement with the GAT. Ocular stiffness is presumed to reflect the properties of the eyeball, IOP, age, gender, and other ocular and systemic factors. Ocular stiffness is defined as the ratio between the change in the force applied to the cornea and the resultant displacement of the corneal apex. The non-invasive experimental system developed by Kurita *et al*. [16] was used to determine ocular stiffness. The GAT was used to apply pressure to the cornea and a high-resolution digital camera was used to measure the displacement of the corneal apex by photographing the cornea in profile. Kurita *et al*. [16] showed, based on mathematical theory and experimentation, that a linear relationship exists between ocular stiffness and IOP_{GAT}. This relationship is here explored further by investigating how ocular stiffness is associated with several clinically obtained parameters, including IOP_{GAT}, and the relationship between ocular stiffness and IOP_{GAT} is quantitatively examined using statistical methods. To our knowledge, this is the first study to measure ocular stiffness using the GAT in living human eyes and to analyze its influence on IOP_{GAT}.

2. Subjects and methods

2.1 Data collection

59 eyes of 59 subjects (33 men and 26 women) were studied. Fifteen of these were diagnosed as having POAG at Hiroshima University Hospital. None of the subjects had received medical treatment to reduce IOP before or during this study. The other 44 subjects were volunteers without any ocular diseases. Subjects with any corneal abnormalities, such as bullous keratopathy, were excluded. The protocol of this study was approved by the Hiroshima University Institutional Review Board (IRB). Before any measurements were made, detailed explanations regarding the goals of the research and the procedures to be used were given to each subject, and informed consent was obtained. All of the procedures conformed to the tenets of the Declaration of Helsinki for research involving human subjects.

The following eight variables were collected for this study: ocular stiffness (K), radius of the anterior surface of the cornea (R), IOP_{GAT}, axial length (AL), anterior chamber depth (ACD), and central corneal thickness (CCT), age (Ag), and gender (G). All data for a subject were collected on the same day, but technical difficulties precluded obtaining complete data for some of the subjects; those data were treated as missing in the relevant analysis.

Ocular stiffness, K [N/m], was defined based on the force-displacement relationship provided by Hooke's law as the ratio between change in the force applied to the cornea by the GAT probe, Δf [N], and the resultant change in displacement of the corneal apex, Δx[m]:

$$K = \Delta f/\Delta x \quad (1)$$

A schematic diagram of the experimental system developed by Kurita *et al*. [16] is shown in Fig. 1. The measurement system consisted of a GAT (900.4.2. HAAG-STREIT; Koeniz, Switzerland) and a high-resolution camera (ADP-210B Flovel Co., Ltd.; Tokyo, Japan). The GAT was used to exert force on the cornea by adjusting the pressure dial. The high-resolution camera was used to measure the displacement of the corneal apex by positioning it perpendicular to the visual axis to photograph the cornea in profile. The camera was capable of photographing images with a size of 1624 × 1234 pixels at 5 Hz (i.e., 5 images per second) with a resolution of 5.6 μm/pixel.

To calculate K in N/m units, pressure (in mmHg) indicated on the dial of the tonometer was converted to force in Newtons, f [N], using the equation developed by Kurita *et al*. [16]:

$$f\,[N] = 0.0011 \times \text{pressure}\,[mmHg]$$

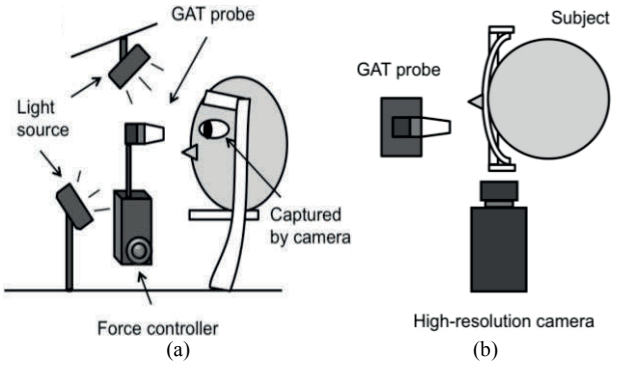

Figure 1. (a) Side view and (b) top view of experimental system. The high-resolution camera was positioned perpendicular to the visual axis to photograph deformation of the corneal apex.

The force applied to the cornea was gradually increased from 0.005 N, the minimum force at which the probe-corneal surface contact can be clearly observed on the digital images (defined as the initial position of the corneal apex), to a level slightly higher than the subjects' IOP_{GAT} (defined as the end position of the corneal apex). It took approximately six seconds to reach the end position from the initial position. The measurement was performed once on each subject according to the IRB-approved protocols.

The corneal surface was manually traced on all photographed images (approximately 30 images per subject), as shown in Fig. 2. To measure corneal apex displacement, the

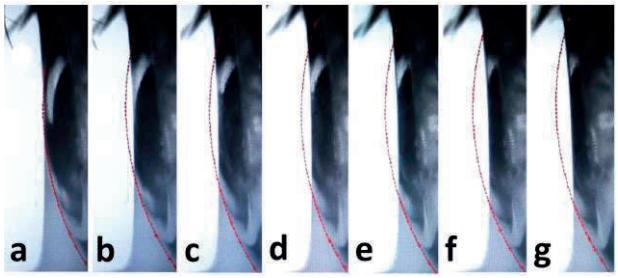

Figure 2. Sequential images of a subject's cornea being applanated by a GAT. Subject's cornea in profile (a) at the initial position (at time zero) and at (b) 1, (c) 2, (d) 3, (e) 4, (f) 5, and (g) 6 seconds after applanation was started. The dotted lines are estimated corneal surface lines obtained by manual tracing.

center of the corneal curvature, which was estimated on all images obtained for each subject based on the position of the points marked along the corneal surface line and the subject's radius of corneal curvature (Fig. 3(a)), was set as a reference point. Corneal apex displacement (x) was calculated based on the geometrical relationship:

$$X = (h + R) - l \quad (2)$$

where h, R, and l are the distance between the line on the probe and the head of the probe, the radius of corneal curvature, and the distance between the line on the probe and the reference point, respectively (Fig. 3(a)). The degree of corneal apex displacement was calculated based on sets of two images (i.e., an image of the initial position and those of all other positions during applanation) for each subject. An image of the cornea and the probe in profile is shown in Fig. 3(b). On all images obtained, the position of the reference point was monitored and the distance between the line on the probe and the reference point (l) was measured. As it was possible that the whole eyeball moved slightly backward while being pressed by the probe, this definition of corneal apex displacement helped ensure that measurements were minimally influenced by changes in eyeball position. The ocular stiffness of each subject was determined by calculating the slope of the least-squares regression line $\Delta f/\Delta x$.

An IOL Master (P10-CZM052 Carl Zeiss Meditec; Jena, Germany) was used to measure the radius of corneal curvature, axial length, and the anterior chamber depth of each eye. The central corneal thickness of each subject was measured with a pachymeter (SP-3000 Tomey Co.; Nagoya, Japan).

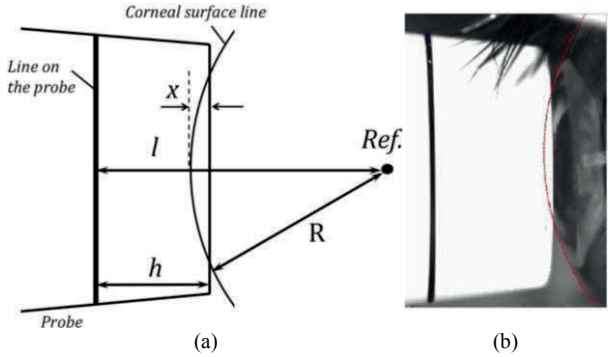

Figure 3. (a) Schematic representation of the parameters used to calculate corneal apex displacement and (b) a real image of the cornea in profile. Based on the radius of corneal curvature and several points marked along the corneal surface line on the images, the reference point (Ref.), i.e., the center of the corneal curvature, was estimated. The distance between the line on the probe and the tip of the probe, the radius of corneal curvature, and the distance between the line on the probe and the reference point, are respectively denoted by h, R, and l.

2.2 Statistical analyses

Three out of the 59 subjects were detected as outliers through least-median-squares regression [17] and were excluded from the analysis. Baseline demographics of the remaining 56 subjects are shown in Table 1.

Table 1. Baseline demographics of the subjects.

Variable	Average	SD [a]	Range	NM [b]
Measured ocular stiffness (K) [N/m]	108	27.5	67.7-197	0
Mean radius of corneal curvature (R) [mm]	7.74	0.264	7.15-8.30	1
GAT-based intraocular pressure (IOP_{GAT}) [mmHg]	16.8	6.00	11.0-41.0	0
Axial length (AL) [mm]	25.3	2.18	22.6-32.8	5
Anterior chamber depth (ACD) [mm]	3.18	0.902	2.00-4.75	7
Central corneal thickness (CCT) [μm]	529	28.2	448-593	8
Age (Ag) [years]	44.7	23.9	19-87	0
Gender[c] (G)	0.560	0.500	0-1	0

[a] SD: standard deviation, [b] NM: number of missing data, [c] Gender was coded for statistical analysis: 0 for women and 1 for men

Simple correlation analyses were used to determine the associations between the eight variables. To enable quantitative analysis, numerical values were assigned to gender (G): 0 for women and 1 for men. Numerical age was used for Ag. The experimental variables measured in millimeters, micrometers, and mmHg were converted to meters or N/m^2. Correlation coefficients were calculated for each pair of variables (twenty-eight combinations).

To investigate how ocular stiffness (K) is related to the other seven variables, the following regression model with K as the response variable and the explanatory variables R, IOP_{GAT}, AL, ACD, CCT, Ag, and G was defined:

$$K = k_1 \bullet U(R, IOP_{GAT}, AL, ACD, CCT, Ag, G) \bullet e^{\varepsilon} \quad (3)$$

where ε is a random error term, including unknown minor factors influencing K, assumed to be normally distributed with

mean zero and variance δ^2, and k_1 is a constant variable. The function U is given by:

$$U = \mathrm{Exp}\ (\beta_1\ \log R+\beta_2\ \log IOP_{GAT}+\beta_3\ \log AL+\beta_4\ \log ACD +\beta_5\ \log CCT+\beta_6\ Ag+\beta_7\ G) \quad (4)$$

with unknown parameter β_x. Exp(x) is the exponential function e^x. Taking the logarithm of Eq. (3) yields the multiple regression model:

$$\log K = \log\ [k_1 \bullet U(R,IOP_{GAT},AL,ACD,CCT,Ag,G) \bullet e^{\varepsilon}] = \beta_0+\beta_1\ \log R+\beta_2\ \log IOP_{GAT}+\beta_3\ \log AL+\beta_4\ \log ACD +\beta_5\ \log CCT+\beta_6\ Ag+\beta_7\ G+\varepsilon \quad (5)$$

Initially, all variables were simultaneously included in the multiple regression model (full model). Subsequently, the Akaike information criteria (AIC) [18] were used to select an optimal model, which represented a combination of variables that best explained K. For each statistical analysis, a *P* value of less than 0.05 was considered significant.

The software used was R version 2.15.2, which implements the extended robust multiple regression algorithm developed by Rousseeuw [17].

3. Results

Correlation coefficients between all variables are shown in Table 2. Preliminary bivariate analysis showed that ocular stiffness was significantly correlated with age ($r = 0.386$, $P = 0.003$) and with IOP_{GAT} ($r = 0.827$, $P < 0.001$). Gender, radius of the anterior surface of the cornea, axial length, anterior chamber depth, and central corneal thickness were not significantly correlated with ocular stiffness (Table 2).

Table 2. Correlations between variables.

	K	R	IOP_{GAT}	AL	ACD	CCT	Ag	G
K	-							
R	0.112	-						
IOP_{GAT}	0.827**	-0.033	-					
AL	0.166	0.198	0.264	-				
ACD	-0.112	0.165	-0.106	0.170	-			
CCT	0.080	0.309*	0.164	0.441**	0.026	-		
Ag	0.386**	-0.289*	0.475**	-0.063	-0.366*	-0.172	-	
G	0.004	0.085	-0.126	-0.195	-0.155	-0.028	0.003	-

**: $p < 0.01$, *: $0.01 \le p < 0.05$

Estimated regression coefficients for each variable in the full model, i.e., the regression model with all variables, are shown in Table 3. AIC/individual sample (AIC/IND) in multiple regression analysis attained its minimum value when the model contained only two explanatory variables, namely log IOP_{GAT} ($P < 0.01$) and log R ($0.05 \le P < 0.1$) (Table 4). In other words, the combination of the radius of corneal curvature and IOP_{GAT} explains ocular stiffness most efficiently. Based on that, the following model equation was specified:

$$E(K) = e^{4.1} \bullet R^{0.92} \bullet (IOP_{GAT})^{0.66} = 60.3 \times R^{0.92} \bullet (IOP_{GAT})^{0.66} \quad (6)$$

where E(K) is the expected value of K.

Applying Eq. (6) to an eye with a corneal radius of curvature of 0.007 m, the relationship between the ocular stiffness and IOP_{GAT} can be simplified to:

$$K = 60.3 \times 0.0104 \times (IOP_{GAT})^{0.66} = 0.627 \times (IOP_{GAT})^{0.66} \quad (7)$$

Therefore:

$$K \propto (IOP_{GAT})^{0.66} \quad (8)$$

Table 3. Estimated regression coefficients of explanatory variables[d] (full model[e]).

Variable	Coefficient	Standard error	*P* value
Constant	0.810	4.30	0.852
log R	1.28[ψ]	0.655	0.0586
log IOP_{GAT}	0.664**	0.101	0.000
log AL	0.216	0.338	0.526
log ACD	-0.131	0.111	0.246
log CCT	-0.661	0.474	0.172
Ag	0.000424	0.00123	0.732
G	0.0313	0.0430	0.472

[f]$\sigma = 0.117$, [g]$R^2 = 0.668$, [h]AIC = - 170, [i] AIC/IND = - 3.94

P values indicate significance of coefficient (**: $p < 0.01$, *: $0.01 \le p < 0.05$, [ψ]: $0.05 \le p < 0.1$)

[d] The response variable is log K, [e] Due to the nature of the full model, subjects with one or more missing data were excluded in the analysis; hence the sample size for this model is 43,

[f] σ: standard deviation of residuals, [g] R^2 : coefficient of determination, [h] AIC: Akaike information criteria, [i] AIC/IND: Akaike information criteria per one sample

Table 4. Estimated regression coefficients of explanatory variables (optimal model [j]).

Variable	Coefficient	Standard error	*P* value
Constant	4.11	2.420	0.096
log R	0.924[ψ]	0.493	0.066
log IOP_{GAT}	0.657**	0.0592	0.000

$\sigma = 0.123$, $R^2 = 0.703$, AIC = - 229, AIC/IND = - 4.09

**: $p < 0.01$, *: $0.01 \le p < 0.05$, [ψ]: $0.05 \le p < 0.1$

[j] Sample size for this model is 56

The relationship between ocular stiffness and IOP_{GAT} is graphed in Fig. 4. This equation shows that a greater change in ocular stiffness is observed as IOP_{GAT} decreases. For example, when IOP_{GAT} drops from 25 mmHg to 24 mmHg, ocular stiffness decreases by 3.52 N/m and $\Delta K/\Delta IOP_{GAT} = 2.62 \times 10^{-2}$. On the other hand, when IOP_{GAT} drops from 15 mmHg to 14 mmHg, ocular stiffness decreases by 4.21 N/m and $\Delta K/\Delta IOP_{GAT} = 3.12 \times 10^{-2}$.

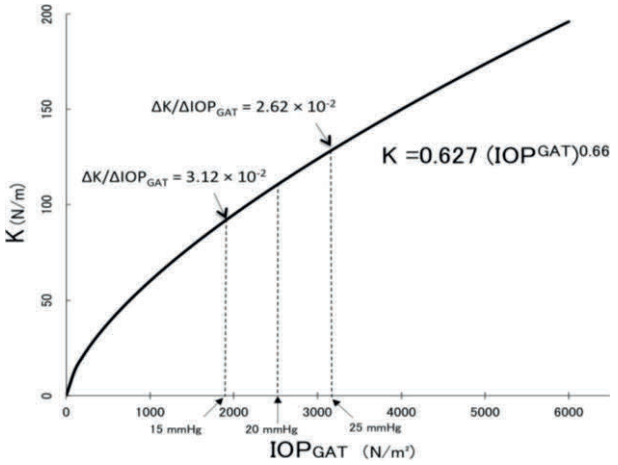

Figure 4. Relationship between ocular stiffness (K) and GAT reading (IOP_{GAT}) of an eye with radius of corneal curvature of 0.007 m. IOP_{GAT} values in N/m^2 equal to 15, 20, and 25 mmHg are indicated on the X axis. The slopes of the curve of the graph are 3.12×10^{-2} and 2.62×10^{-2} at IOP_{GAT} values of 15 and at 25 mmHg, respectively.

4. Discussion

Ocular stiffness is here considered to be an important biomechanical factor influencing corneal deformation during IOP measurement by a GAT. In this study, ocular stiffness was defined as the ratio between the change in the force applied to the cornea by a GAT probe and the resultant displacement of the corneal apex. To measure the amount of corneal apex displacement, the cornea was photographed in profile using a high-resolution camera during tonometry. Then, the association of ocular stiffness with clinical parameters, including GAT readings was investigated and the relationship between ocular stiffness and IOP_{GAT} was assessed.

Past studies have used various terminology regarding ocular biomechanical properties, such as elasticity, hysteresis, rigidity, and stiffness, to describe specific aspects of ocular deformation by an applied force. The definition used for a certain term also varies between studies. Given that variability, the present study has stayed true to the original papers by using their respective terminologies exactly.

Several studies based on different methods have suggested that IOP and corneal elasticity are related [11-13,15]. For example, Orssengo and Pye [11] reported that the elastic corneal modulus is proportional to the estimated IOP. Later, Hamilton and Pye [13] used the Orssengo-Pye algorithm to calculate corneal Young's modulus and reported a positive relationship between the theoretical error in IOP_{GAT} and corneal Young's modulus. Liu and Roberts [12] showed mathematically that a change in corneal Young's modulus corresponded with changes in IOP. Furthermore, Elsheikh *et al*. [19] reported similar results after examining the relationship between the corneal modulus of elasticity and the force applied to the cornea from the endothelial side.

In contrast, only a small number of studies have examined ocular rigidity/stiffness and its relation to IOP. Among those, Hommer *et al*. [15] measured changes in the volume of the eye and IOP caused by rhythmic filling of the intraocular vessels during a cardiac cycle using laser interferometry and pneumotonometry. Based on Friedenwald's formula, they defined the rigidity of the eye as the ratio of the change in IOP to the fluctuation of ocular volume, and demonstrated that ocular rigidity was significantly higher in eyes with hypertensive POAG than that in normal eyes. Kurita *et al*. [16] showed that there was a linear relationship between ocular stiffness and IOP_{GAT}.

While our findings agree with their result [15,16], our study adds further quantitative insights into the relationship by incorporating additional clinical parameters in the analysis. Our analysis shows that ocular stiffness is best explained by a unique quantitative combination of IOP_{GAT} and the radius of corneal curvature (R), shown in Eq. (6). Applying the equation to an eye, IOP_{GAT} became the sole factor explaining the ocular stiffness, shown in Eq. (7). This demonstrates that ocular stiffness is not linearly related to IOP_{GAT} but proportionately related to $(IOP_{GAT})^{0.66}$. In other words, ocular stiffness decreases progressively more as IOP_{GAT} becomes lower, especially in the normal range of IOP. For example, as shown in Fig. 4, when the IOP_{GAT} drops from 25 to 20 mmHg, from 20 to 15 mmHg, and from 15 to 10 mmHg, ocular stiffness decreases 18.14, 19.78, and 22.2 N/m, respectively.

Ocular stiffness is presumed to be affected by many factors, such as true IOP, structure of the eyeball, flexibility of the cornea and sclera, properties of the uvea and vitreous body, viscosity of the aqueous humor, and the surroundings of the eyeball including tear film. Among the above mentioned factors, true IOP is the only one that changes over a short period. Thus, change in true IOP is considered to be the primary cause influencing change in ocular stiffness. Therefore, it is possible that in the lower IOP range, true IOP drops to a greater extent than the value indicated by IOP_{GAT}. In other words, treatment outcome for eyes with lower pre-treatment IOP_{GAT} would be considered better than those with higher pre-treatment IOP_{GAT} for a given reduction in IOP_{GAT}. Therefore, the clinical implication of our findings is that the effectiveness of glaucoma treatment should be interpreted differently depending on the IOP_{GAT} recorded prior to commencement of treatment.

Kniestedt *et al*. [20] measured the difference in value between GAT-based IOP and dynamic contour tonometer (DCT)-based IOP, which is considered to be closer to true IOP, on enucleated human cadaver eyes. The authors reported that DCT-based IOP was progressively higher than IOP_{GAT} as IOP increased, i.e., rate of change in DCT-based IOP was greater than that of GAT-based IOP [20]. Our results agree with their data, and we suspect that ocular stiffness underlies the difference between the two IOP values (GAT-based and DCT-based). Thus, development of more reliable and practical instruments for IOP measurement in which ocular stiffness is considered, e.g., improved DCT, is desirable.

To establish an equation for converting IOP_{GAT} into the true IOP value, IOP must be measured simultaneously by GAT and a pressure sensor inserted into the eyeball in the operating room, which is highly invasive to the eye and presents obvious practical and ethical obstacles for study. Therefore, even though our study does not yet allow prediction of the true IOP value from IOP_{GAT}, our results have clinical implications, particularly in terms of assessing the effectiveness of treatment in eyes with normal-tension glaucoma, which is highly prevalent in the Japanese population [21].

In addition, while our univariate analysis demonstrated a significant correlation between age and ocular stiffness ($r = 0.386$, $P = 0.003$) (Table 2), this correlation was not significant in the multivariate regression analysis (Tables 3 and 4). The cause of this discrepancy may be that, although both age and IOP_{GAT} were correlated with ocular stiffness, the relatively much stronger relationship between IOP_{GAT} and ocular stiffness may have masked the relationship between age and ocular stiffness in the multivariate regression analysis. To untangle this discrepancy, the data were stratified by IOP_{GAT} (high vs. low IOP_{GAT}) using median IOP (15 mmHg) as the cut-off value. In the lower IOP_{GAT} group, there was no correlation between ocular stiffness and age ($r = 0.087$, $P = 0.65$), but in the group with higher IOP_{GAT} there was a suggestive correlation between ocular stiffness and age ($r = 0.32$, $P = 0.1$).

These relationships are graphed in Fig. 5. Although age was not selected as an explanatory factor for ocular stiffness in the multiple regression analysis, it is nevertheless possible that age had some influence on ocular stiffness in high-IOP eyes.

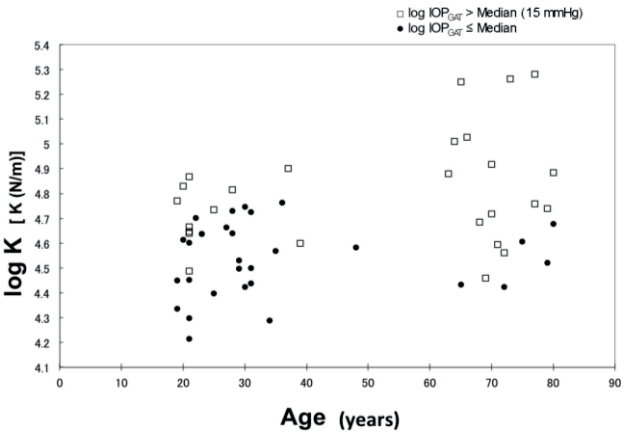

Figure 5. Relationship between ocular stiffness and age. Ocular stiffness (K) was significantly correlated with age (r = 0.386, P = 0.003). In the high-IOP$_{GAT}$ group (squares), the correlation was suggestive (r = 0.32, P = 0.1). There was no correlation between the two variables in the low-IOP$_{GAT}$ group (shaded circles) (r = 0.087, P = 0.65).

The correlation between age and corneal biomechanical properties has been shown in several studies [19,22,23], but only a small number of studies have investigated the link between age and ocular biomechanical properties. One such study was done by Pallikaris *et al*. [24]. In their study, ocular rigidity was calculated as the slope of the pressure versus volume curve by examining how much IOP increased after a designated volume of saline solution had been injected into the eye. They found a positive correlation between age and ocular rigidity, which is comparable to our results. Although it is difficult to draw a definitive conclusion about the association between age and ocular stiffness, the following offers a possible biological explanation for this association. It has been shown that the number of cross-links between collagen fibers caused by glycation increases with increasing age [25,26]. Aging has also been linked to degeneration of proteoglycans in the corneal stroma that would widen the space between collagen molecules [27,28]. Therefore, such age-related degeneration of the connective tissue is a possible mechanism underlying the association found here.

Gender, anterior chamber depth, axial length, and central corneal thickness were not significantly associated with ocular stiffness in our study. This lack of association between these variables and the ocular stiffness is consistent with findings in the literature. For instance, Pallikaris *et al*. [24] reported that neither axial length nor corneal thickness was significantly associated with ocular rigidity. While studies that examined the influence of the central corneal thickness on corneal biomechanical properties have reported mixed results [22,23], Other study [29] reported that central corneal thickness did not explain differences in the DCT-based and GAT-based values of IOP, pointing to the need to consider ocular biomechanical properties in IOP evaluation.

There are several limitations to our study. First, our experimental system is based on the assumption that the reference point is fixed and immobile throughout the experiment. However, it is possible that applanation deforms not only the area of the cornea that comes in contact with the probe but also its periphery (Fig. 6). This could result in inaccuracy in the estimation of the reference point (Ref. in Fig. 6), which would in turn induce a small error in the measurement of corneal displacement. However, as best as we could observe, deformation of the cornea outside the applanated area was not present in the images; hence, the amount of such error in corneal displacement measurement should be small.

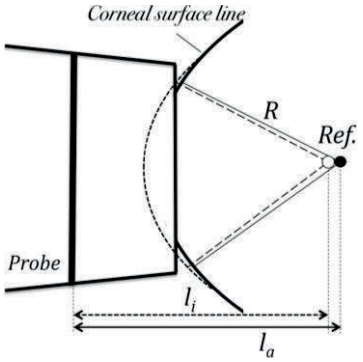

Figure 6. Mechanism of possible error in the determination of corneal apex displacement. It is possible that applanation deformed the applanated area as well as the area peripheral to it (thick solid curved line), in which case the position of the reference point (Ref.) would have been estimated to exist deeper in the eye (black circle) than it actually did (white circle). This potential inaccuracy in estimation of the reference point may have caused an error in the determination of corneal displacement (l_a - l_i), where l_i represents the distance between the line on the probe and the reference point determined when the probe was in its initial position, and l_a is the corresponding value during applanation. The radius of the corneal curvature is denoted by R. The dotted curved line indicates the corneal surface line of the initial position.

A second limitation is that the measurement was performed only once on each subject. Repeated measurements would be desirable because they allow decomposition of the random error into individual variation and measurement error. However, the IRB protocol allowed only one measurement per subject to minimize inconvenience and potential harm to subjects.

The main strength of this study is that the experiment was performed non-invasively on living human eyes. Because all measurements were made without systemic or retrobulbar anesthesia, the data were not affected by muscle relaxation or paralysis of the nerves. Also, the results were not affected by postmortem changes, i.e., there was no rigor mortis or edema in the ocular tissues including the muscles and blood vessels. Hence, the results are expected to reflect the natural condition of human eyes.

5. Conclusion

This study investigated how ocular stiffness is related to

clinical parameters including IOP_{GAT}. Ocular stiffness was defined as the ratio between the change in force applied to the cornea by a GAT and the resulting displacement of the corneal apex. To measure ocular stiffness, the corneal apex displacement was photographed during applanation in living human eyes and multiple regression analysis was used to investigate how ocular stiffness is associated with clinical parameters. Ocular stiffness was found to be proportional to the product of $(IOP_{GAT})^{0.66}$ and $(R)^{0.92}$. In individual eyes, ocular stiffness was proportionately related to $(IOP_{GAT})^{0.66}$. This relationship means that the extent of ocular stiffness decreases progressively more as IOP_{GAT} becomes lower. Among many factors presumed to affect ocular stiffness, true IOP is the only factor that changes over a short period. Thus, the change in true IOP is considered to be the primary cause influencing change in ocular stiffness. Therefore, it is possible that as IOP diminishes, the range of true IOP drop is more extensive than indicated by IOP_{GAT}. This suggests that, in patients with lower IOP_{GAT}, the true IOP drop with treatment may become progressively greater than the value indicated by IOP_{GAT}.

Given that inserting a pressure sensor into the eye is not a clinically feasible method to measure true IOP, our findings have important practical implications for the management of glaucoma in ordinary clinical settings where a GAT is used to measure IOP, by helping to improve the accuracy of IOP estimation needed to evaluate the effectiveness of glaucoma treatment.

Acknowledgments

We gratefully acknowledge Professor Megu Ohtaki, Department of Environmetrics and Biometrics, Research Institute for Radiation Biology and Medicine, Hiroshima University, for his assistance with the statistical analyses.

References

[1] H. Goldmann, "Applanation tonometry," *Glaucoma: Transactions of the Second Conference*, 167-220, 1957.

[2] Y. Harada, N. Hirose, T. Kubota and A. Tawara, "The influence of central corneal thickness and corneal curvature radius on the intraocular pressure as measured by different tonometers: noncontact and goldmann applanation tonometers," *J. Glaucoma*, 17: 619-625, 2008.

[3] M. M. Whitacre, R. A. Stein and K. Hassanein, "The effect of corneal thickness on applanation tonometry," *Am. J. Ophthalmol.*, 115: 592-596, 1993.

[4] A. Browning, A. Bhan, A. Rotchford, S. Shah and H. Dua, "The effect of corneal thickness on intraocular pressure measurement in patients with corneal pathology," *Br. J. Ophthalmol.*, 88: 1395-1399, 2004.

[5] S. Shah, A. Chatterjee, M. Mathai, S. P. Kelly, J. Kwartz, D. Henson and D. McLeod, "Relationship between corneal thickness and measured intraocular pressure in a general ophthalmology clinic," *Ophthalmology*, 106: 2154-2160, 1999.

[6] Y. Morad, E. Sharon, L. Hefetz and P. Nemet, "Corneal thickness and curvature in normal-tension glaucoma," *Am. J. Ophthalmol.*, 125: 164-168, 1998.

[7] M. Shimmyo, A. J. Ross, A. Moy and R. Mostafavi, "Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics, and African Americans," *Am. J. Ophthalmol.*, 136: 603-613, 2003.

[8] Y. C. Ko, C. J. Liu and W. M. Hsu, "Varying effects of corneal thickness on intraocular pressure measurements with different tonometers," *Eye*, 19: 327-332, 2005.

[9] A. Tomlinson and C. I. Phillips, "Applanation tension and axial length of the eyeball," *Br. J. Ophthalmol.*, 54: 548-553, 1970.

[10] O. Pärssinen, "Intraocular pressure in school myopia," *Acta Ophthalmol.*, 68: 559-563, 1990.

[11] G. J. Orssengo and D. C. Pye, "Determination of the true intraocular pressure and modulus of elasticity of the human cornea in vivo," *Bull. Math. Biol.*, 61: 551-572, 1999.

[12] J. Liu and C. J. Roberts, "Influence of corneal biomechanical properties on intraocular pressure measurement: quantitative analysis," *J. Cataract Refract. Surg.*, 31: 146-155, 2005.

[13] K. Hamilton and D. Pye, "Young's modulus in normal corneas and the effect on applanation tonometry," *Optom. Vis. Sci.*, 85: 445-450, 2008.

[14] Y. Kiuchi, M. Kaneko, H. Mochizuki, J. Takenaka, K. Yamada and J. Tanaka, "Corneal displacement during tonometry with a noncontact tonometer," *Jpn. J. Ophthalmol.*, 56: 273-279, 2012.

[15] A. Hommer, G. Fuchsjäger-Mayrl, H. Resch, C. Vass, G. Garhofer and L. Schmetterer, "Estimation of ocular rigidity based on measurement of pulse amplitude using pneumotonometry and fundus pulse using laser interferometry in glaucoma," *Invest. Ophthalmol. Vis. Sci.*, 49: 4046-4050, 2008.

[16] Y. Kurita, R. Kempf, Y. Iida, J. Okude, M. Kaneko, H. K. Mishima, H. Tsukamoto, E. Sugimoto, S. Katakura, K. Kobayashi and Y. Kiuchi, "Contact-based stiffness sensing of human eye," *IEEE Trans. Biomed. Eng.*, 55: 739-745, 2008.

[17] P. J. Rousseeuw, "Least median of squares regression," *J. Am. Statist. Assoc.*, 79: 871-880, 1984.

[18] H. Akaike, "Information theory and an extension of the maximum likelihood principle," *Second International Symposium on Information Theory*, 1: 267-281, 1973.

[19] A. Elsheikh, D. Wang and D. Pye, "Determination of the modulus of elasticity of the human cornea," *J. Refract. Surg.*, 23: 808-818, 2007.

[20] C. Kniestedt, M. Nee and R. L. Stamper, "Dynamic contour tonometry: A comparative study on human cadaver eyes," *Arch. Ophthalmol.*, 122: 1287-1293, 2004.

[21] A. Iwase, Y. Suzuki, M. Araie, T. Yamamoto, H. Abe, S. Shirato, Y. Kuwayama, H. Mishima, H. Shimizu, G. Tomita, Y. Inoue and Y. Kitazawa, "The prevalence of primary open-angle glaucoma in Japanese: the Tajimi Study," *Ophthalmology*, 111: 1641-1648, 2004.

[22] A. Kotecha, A. Elsheikh, C. R. Roberts, H. Zhu and D. F. Garway-Heath, "Corneal thickness- and age-related biomechanical properties of the cornea measured with the ocular response analyzer," *Invest. Ophthalmol. Vis. Sci.*, 47: 5337-5347, 2006.

[23] K. Kamiya, K. Shimizu and F. Ohmoto, "Effect of aging on corneal biomechanical parameters using the ocular response analyzer," *J. Refract. Surg.*, 25: 888-893, 2009.

[24] I. G. Pallikaris, G. D. Kymionis, H. S. Ginis, G. A. Kounis and M. K. Tsilimbaris, "Ocular rigidity in living human eyes," *Invest. Ophthalmol. Vis. Sci.*, 46: 409-414, 2005.

[25] S. Ricard-Blum and G. Ville, "Collagen cross-linking," *Int. J. Biochem.*, 21: 1185-1189, 1989.

[26] R. C. Siegel, "Lysyl oxidase," *Int. Rev. Connect. Tissue Res.*, 8: 73-118, 1979.

[27] N. S. Malik, S. J. Moss, N. Ahmed, A. J. Furth, R. S. Wall and K. M. Meek, "Ageing of the human corneal stroma: structural and biochemical changes," *Biochim. Biophys. Acta*, 1138: 222-228, 1992.

[28] A. Kanai and H. E. Kaufman, "Electron microscopic studies of corneal stroma: aging changes of collagen fibers," *Ann. Ophthalmol.*, 5: 285-287, 1973.

[29] E. Schneider and F. Grehn, "Intraocular pressure measurement comparison of dynamic contour tonometry and Goldmann applanation tonometry," *J. Glaucoma*, 15: 2-6, 2006.