



저작자표시 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.
- 이차적 저작물을 작성할 수 있습니다.
- 이 저작물을 영리 목적으로 이용할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#) 

A DISSERTATION
FOR THE DEGREE OF MASTER

**Comparison of Corneal Thickness Measurements
using Ultrasound Pachymetry, Ultrasound
Biomicroscopy, and Digital Caliper
in Frozen Canine Corneas**

개의 냉동 각막에서 초음파각막두께측정계,
초음파생체현미경과 디지털캘리퍼를 이용한
각막 두께 측정법의 비교

by
Seowo Jeong

MAJOR IN VETERINARY CLINICAL SCIENCE
DEPARTMENT OF VETERINARY MEDICINE
GRADUATE SCHOOL
SEOUL NATIONAL UNIVERSITY

August, 2017

**Comparison of Corneal Thickness Measurements
using Ultrasound Pachymetry, Ultrasound
Biomicroscopy, and Digital Caliper
in Frozen Canine Corneas**

by

Seowoo Jeong

Supervised by

Professor Kangmoon Seo

Thesis

Submitted to the Faculty of the Graduate School
of Seoul National University
in partial fulfillment of the requirements
for the Degree of Master
in Veterinary Medicine

April, 2017

Major in Veterinary Clinical Science
Department of Veterinary Medicine
Graduate School
Seoul National University

June, 2017

**Comparison of Corneal Thickness Measurements
using Ultrasound Pachymetry, Ultrasound
Biomicroscopy, and Digital Caliper
in Frozen Canine Corneas**

개의 냉동 각막에서 초음파각막두께측정계,
초음파생체현미경과 디지털캘리퍼를 이용한
각막 두께 측정법의 비교

지도교수 서 강 문

이 논문을 수의학 석사 학위논문으로 제출함
2017 년 04 월

서울대학교 대학원
수 의 학 과 임 상 수 의 학 전 공
정 서 우

정서우의 석사학위논문을 인준함
2017 년 06 월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

**Comparison of Corneal Thickness Measurements
using Ultrasound Pachymetry, Ultrasound
Biomicroscopy, and Digital Caliper
in Frozen Canine Corneas**

Supervised by

Professor Kangmoon Seo

Seowoo Jeong

Major in Veterinary Clinical Sciences, Department of Veterinary Medicine

Graduate School,

Seoul National University

ABSTRACT

The purpose of this study was to evaluate the clinical efficacy among ultrasound pachymetry (UP), ultrasound biomicroscopy (UBM), and manual measurement (MM) with digital caliper by comparing corneal thickness (COT) values obtained on frozen canine corneas *ex vivo*. The COT was measured using UP, UBM, and MM in 8 enucleated normal canine eyes frozen at -20°C for 4 weeks. After thawing at room temperature for 2 hrs, the COT values were obtained from five sites in each cornea: central corneal thickness (CCOT), thickness at the 3, 6, 9, and 12 o'clock positions

(3COT, 6COT, 9COT, and 12COT, respectively). For each device, measurements were performed three times at each designated site by one operator. The mean CCOT was 839.0 ± 138.2 , 857.6 ± 127.9 , and 849.1 ± 132.8 μm for UP, UBM, and MM, respectively. There was no statistically significant difference among the measurements by all three devices. The statistical agreement among these devices was also shown to be good according to Bland-Altman plots. In addition, the values measured on the peripheral sites of the cornea (3COT, 6COT, 9COT, and 12COT) also revealed no significant differences among the three devices. The difference between CCOT and peripheral corneal thickness (PCOT) was also statistically insignificant. Based on the results of the present study, the UP, UBM, and MM measurements showed statistically similar COT values. The UBM and UP provided accurate and reliable measurements comparable to MM, and could be used effectively for clinical COT measurements.

Key words: corneal thickness, frozen cornea, ultrasound pachymetry, ultrasound biomicroscopy, dog

Student number: 2015-23165

List of Abbreviations

| | |
|---------------|---|
| ANOVA | Analysis of variance |
| AS-OCT | Anterior segment optical coherence tomography |
| CI | Confidence interval |
| CCOT | Central corneal thickness |
| COT | Corneal thickness |
| ICC | Intraclass correlation coefficient |
| LL | Limbus to limbus |
| LoA | Limits of agreement |
| MM | Manual measurement |
| PCOT | Peripheral corneal thickness |
| SD | Standard deviation |
| UBM | Ultrasound biomicroscopy |
| UP | Ultrasound pachymetry |
| 3COT | Corneal thickness of 3 o'clock position |
| 6COT | Corneal thickness of 6 o'clock position |
| 9COT | Corneal thickness of 9 o'clock position |
| 12COT | Corneal thickness of 12 o'clock position |

CONTENTS

| | |
|---|----|
| Introduction | 1 |
| Materials and Methods | 3 |
| 1. Preparation of frozen canine eyes | 3 |
| 2. General procedures | 4 |
| 3. Measurement using ultrasound pachymetry | 6 |
| 4. Measurement using ultrasound biomicroscopy | 7 |
| 5. Manual measurement using digital capliper | 9 |
| 6. Statistical Analyses | 11 |
| Results | 12 |
| Discussion | 19 |
| Conclusions | 24 |
| References | 25 |
| Abstract in Korean | 30 |

Introduction

Accurate measurement of corneal thickness (COT) is essential to evaluate ocular health. It is an important parameter for diagnosing of ophthalmic diseases, planning surgical procedures, and monitoring of treatment response (Alario and Pirie, 2014). The COT has also been known to influence accurate interpretation of intraocular pressure by tonometry (Park *et al.*, 2011).

In veterinary ophthalmology, several surgical methods have been used for treatment of corneal perforation or deep corneal defects including conjunctival grafts, amniotic and renal membranes, frozen lamellar corneal grafting (Hansen and Guandalini, 1999) and full-thickness corneal grafting (Lacerda *et al.*, 2016). Among these procedures, frozen corneal grafting requires an accurate measurement of COT of the frozen cornea.

Many devices have been used to measure COT, including ultrasound pachymetry (UP, Gilger *et al.*, 1991), optical coherence tomography (OCT, Alario and Pirie, 2014; Strom *et al.*, 2016), specular microscopy (Tam and Rootman, 2003), and ultrasound biomicroscopy (UBM, Al-Farhan and Al-Otaibi, 2012; Tam and Rootman, 2003). Currently, the UP is the most commonly used device for measuring COT in veterinary ophthalmology. UP uses ultrasound waves to detect reflections from the epithelial and endothelial surfaces of the cornea. The COT is measured by calculating the time difference of detection between the two reflections. The measurement of UP is objective, and demonstrated to have high intraobserver reproducibility (Gilger *et al.*, 1991; 1993). However, a separate study reported that interobserver reproducibility was low (Bovelle *et al.*, 1999).

The use of UBM to measure various values in the anterior segment of the globe has been reported in veterinary ophthalmology (Urbak, 1998). It has been shown that the accuracy of various measurements by UBM is high (Urbak, 1998). Intraobserver reproducibility has been found to be high for measurements of COT by UBM (Urbak *et al.*, 1998). Although new devices such as anterior segment optical coherence tomography (AS-OCT) have been introduced and also provide enhanced images than UBM, previous studies have shown that there was no statistically significant difference between the two measuring devices (Dada *et al.*, 2007). The measurements using UBM show low interobserver reproducibility because of subjective interpretation of the image (Urbak *et al.*, 1998).

As the use of UBM increases in veterinary ophthalmology, verification of the reliability of the measurements in each situation is also required. Therefore, the objective of the present study was to evaluate the clinical efficacy of UP, UBM, and manual measurement (MM) with digital caliper in measuring COT values of frozen canine corneas *ex vivo*.

Materials and Methods

1. Preparation of frozen canine eyes

Eight frozen enucleated eyes were used for this study. The eyes were obtained from 4 beagle dogs without ocular disease that were euthanized for other research purposes. The eyes were enucleated by routine subconjunctival technique in sterile condition immediately after euthanasia. Each eye was frozen in a sterilized container with a gauze soaked in tobramycin (Ocuracin eye drops®; Samil pharm Co., Ltd, Seoul, South Korea) at -20°C for 4 weeks. Preservation of the eyes was performed according to the previous study (Lacerda *et al.*, 2016). Before measuring the COT, frozen eyes were defrosted for 2 hrs at room temperature. This study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-170103-7).

2. General procedures

In all eyes, measurements were performed first by UP, then UBM, and finally MM. While being measured, the eyes were regularly received artificial tears (Refresh Plus®; Allergan, Irvine, CA, USA) to minimize dryness of the cornea and influence of the previous measurement. Corneal thickness was measured at five sites in each cornea (Fig. 1): central corneal thickness (CCOT), thickness at the 3, 6, 9, and 12 o'clock positions (3COT, 6COT, 9COT, and 12COT, respectively). Before the measurements, all eyes were marked using a surgical pen at the 12 o'clock position of the eyeball to determine the exact direction of the globe. Moreover, the length of the cornea was determined by measuring the distance from one side of the limbus to the opposite side, so exactly the same spot could be checked with all three devices. All measurements of the COT were performed three times by the same operator at each designated site and the mean values were used for statistical analysis.

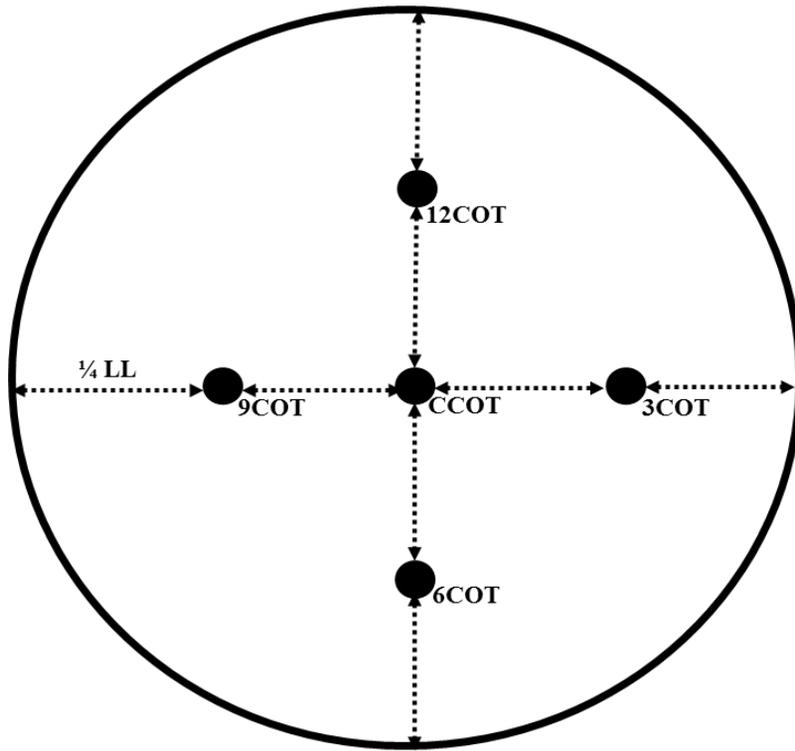


Fig. 1. Schematic diagrams of the frozen cornea. The round solid line indicated limbus of cornea and the measurement sites were represented by black dots. The dotted arrows indicated $\frac{1}{4}$ values of distance from limbus to limbus. LL, limbus to limbus; CCOT, central corneal thickness; 3COT, corneal thickness of 3 o'clock position; 6COT, corneal thickness of 6 o'clock position; 9COT, corneal thickness of 9 o'clock position; 12COT, corneal thickness of 12 o'clock position.

3. Measurement using ultrasound pachymetry

Prior to the measurement, the ultrasound pachymeter (Pachmate DGH; DGH Technology Inc., PA, USA) was precalibrated. The probe of the UP was aligned perpendicular to five designated sites of the cornea and placed gently on the surface of the cornea. Pachymetry values on the display were represented as the average and standard deviation (SD) of 25 separate readings. The values were accepted only if the SD of the measurement was $< 10 \mu\text{m}$.

4. Measurement using ultrasound biomicroscopy

After measurement by UP, each eye was imaged using UBM with a 50-MHz probe (MD-320W; Meda Co., Ltd, Tianjin, China). To evaluate CCOT, the transducer was placed perpendicular to the center of the cornea. Centrality was confirmed by obtaining an image in which the pupil diameter was largest. Values of peripheral corneal thickness (PCOT) were measured in separate images when the transducer was located perpendicular to each limbus at the 3, 6, 9, and 12 o'clock positions of the eye. While scanning, several images were saved and the best 3 images of each eye were used for analysis. All values of COT were measured using the internal caliper included in the UBM software. In each image, the CCOT and PCOT were measured three times. The CCOT was defined as the distance from the anterior to the posterior sites at the brightest reflection peaks of the central cornea (Fig. 2a). PCOT was defined as a value measured perpendicularly to spots at a distance of one quarter of corneal diameter from the edge of the limbus (Fig. 2b).

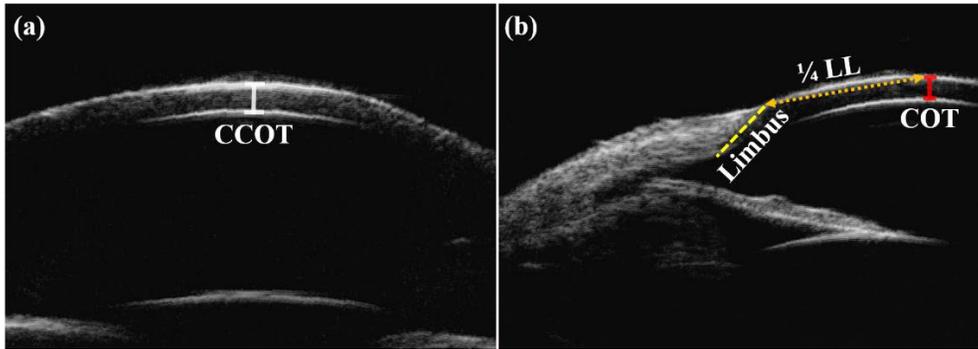


Fig. 2. The ultrasound biomicroscopy (UBM) images for measuring COT in frozen cornea of normal dog. (a) Image in central plane for measuring CCOT. (b) Image in the sagittal plane for measuring COT at 3, 6, 9, and 12 o'clock positions of each cornea. UBM, ultrasound biomicroscope; COT, corneal thickness; CCOT, central corneal thickness; LL, limbus to limbus.

5. Manual measurement using digital caliper

The MMs were made for each spot with a digital caliper (CD-15PS calipers; Mitutoyo Co., Kanagawa, Japan) with a resolution of 0.01 mm under a binocular magnifier with a 3.5X lens (Opti-Visor; Donegan optical Co., KS, USA). The measurement procedure was as follows. The cornea was carefully separated from the globe (Figs. 3a and 3b), taking care not to damage the cornea. The cornea was then bisected using a microtome knife along the 12 o'clock pre-marked site (Fig. 3c). A digital caliper was then used to measure the thickness of the cornea at the midpoint of the cutting plane. The cornea was placed between the caliper tips and the COT was recorded at the point where minimal tension occurred (Fig. 3d). Finally, the center of the bisected cornea was cut again and divided into quarter segments (Fig. 3e). The COT was measured using the previously described process. A total of five measurements were recorded for each cornea.

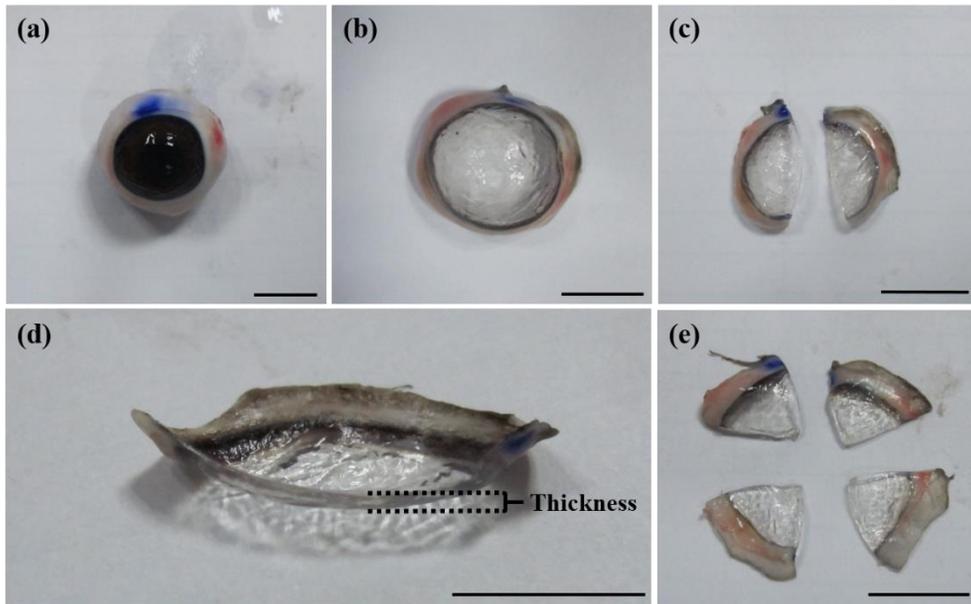


Fig. 3. Procedures of manual measurement using digital caliper.

(a) The canine frozen eye defrosted for 2 hours at room temperature. (b) The cornea separated from the globe. (c) The cornea bisected with a microtome knife. (d) The technique of measurement, the cornea was placed between the caliper tips, and the value of measurement was recorded at the point where minimal tension occurred (dotted line). (e) The center of the bisected cornea was cut again and divided into quarter segments and COT was measured at the point. Scale bar = 1 cm.

6. Statistical analyses

All statistical analyses were performed using the SPSS Statistics V20.0 (SPSS, Inc., Chicago, IL, USA). To test repeatability of measurements in each device, the intraclass correlation coefficient (ICC) was calculated on each instrument. One-way analysis of variance (ANOVA) was conducted to compare the mean values of COT for all three devices. This comparison was conducted separately for central site, 3, 6, 9, and 12 o'clock sites of each cornea. The agreement between each pair of devices was investigated using Bland-Altman analysis. The mean value of these differences was represented by the average bias. The upper and lower limits of agreement (LoA) showed 95% confidence intervals for the mean differences. The ICC values greater than 0.900 were considered to represent a good agreement, and P value < 0.05 was considered statistically significant for each comparison.

Results

The mean COT and 95% confidence interval (CI) for the five sites of each eye measured from UP, UBM, and MM are shown in Table 1. Before comparing the mean values among the devices, the difference between the CCOT and PCOT was calculated. Consequently, the central cornea was thicker on average than the peripheral cornea in all designated sites. However, the differences between CCOT and PCOT were not significantly different among all three devices. Each value measured on the peripheral sites of the 3COT, 6COT, 9COT, and 12COT also showed no significant difference among the three devices. Because there was no statistically significant difference in all locations and among devices, the comparison of these devices was only performed in the central cornea.

The values of ICC to evaluate intraobserver reproducibility of measurements using each device were 0.998, 0.975, and 0.995 for UP, UBM, and MM, respectively. Good intraobserver reproducibility was demonstrated in the devices because all values of ICC were higher than 0.900.

The mean CCOT was 839.0 ± 138.2 , 857.6 ± 127.9 , and 849.1 ± 132.8 μm for UP, UBM, and MM, respectively, and there was no statistically significant difference among the measurements by the three devices (Table 1). In the 95% limits of agreement (LoA) analysis between UP and UBM, between UP and MM, and between UBM and MM at center, the ranges were between -43.9 μm and 6.6 μm , between -32.7 μm and 12.5 μm , and between -10.1 μm and 27.1 μm , respectively (Table 2). Therefore, the difference between upper and lower 95% LoA was 50.5 μm , 45.2 μm , and 37.2 μm , respectively (Table 2). The Bland-Altman plots

demonstrated modalities of random scattered points of the difference in mean CCOT values measured by each device. The agreement between UP and UBM, between UP and MM, and between UBM and MM was represented by Bland-Altman plot with 95% LoA analysis (Figs. 4-6). All of the values were within the 95% LoA for the mean difference.

Table 1. Corneal thickness measured by ultrasound pachymetry, ultrasound biomicroscopy, and manual measurement

| Corneal location | COT (μm) | | | 95% CI (μm) | | |
|------------------|-----------------------|-------------------|-------------------|--------------------------|-------------|-------------|
| | UP | UBM | MM | UP | UBM | MM |
| CCOT | 839.0 \pm 138.2 | 857.6 \pm 127.9 | 849.1 \pm 132.8 | 723.4-954.6 | 750.7-964.5 | 738.1-960.1 |
| 3COT | 815.8 \pm 139.7 | 843.3 \pm 124.8 | 834.1 \pm 129.4 | 698.9-932.5 | 739.1-947.6 | 725.9-942.3 |
| 6COT | 812.6 \pm 143.7 | 840.5 \pm 130.6 | 831.9 \pm 125.8 | 692.5-932.7 | 731.4-949.7 | 726.7-937.1 |
| 9COT | 815.8 \pm 148.5 | 842.0 \pm 128.8 | 830.0 \pm 127.1 | 691.7-939.9 | 734.3-949.7 | 723.8-936.3 |
| 12COT | 820.3 \pm 132.9 | 836.2 \pm 129.5 | 833.2 \pm 129.5 | 709.2-931.5 | 733.4-938.6 | 724.9-941.5 |

Data was presented as Mean \pm SD; CI, confidence interval; COT, corneal thickness; UP, ultrasound pachymetry; UBM, ultrasound biomicroscopy; MM, manual measurement; CCOT, 3COT, 6COT, 9COT, and 12COT, corneal thickness of central, 3 o'clock, 6 o'clock, 9 o'clock, and 12 o'clock position

Table 2. The mean differences of central corneal thickness, including 95% limits of agreement among three devices

| | Mean difference (μm) * | Width of 95% LoA (μm) † | Rate of difference (%) |
|-----------|-------------------------------------|--------------------------------------|------------------------|
| UP vs UBM | -18.6 ± 12.6 | 50.5 (-43.9-6.6) | 5.9 |
| UP vs MM | -10.1 ± 11.3 | 45.2 (-32.7-12.5) | 5.3 |
| UBM vs MM | 8.6 ± 9.3 | 37.3 (-10.1-27.1) | 4.3 |

LoA, limits of agreement; CCOT, central corneal thickness; UP, ultrasound pachymetry; UBM, ultrasound biomicroscopy; MM, manual measurement. * Data was presented as Mean \pm SD. † Data was presented as Width (lower-upper).

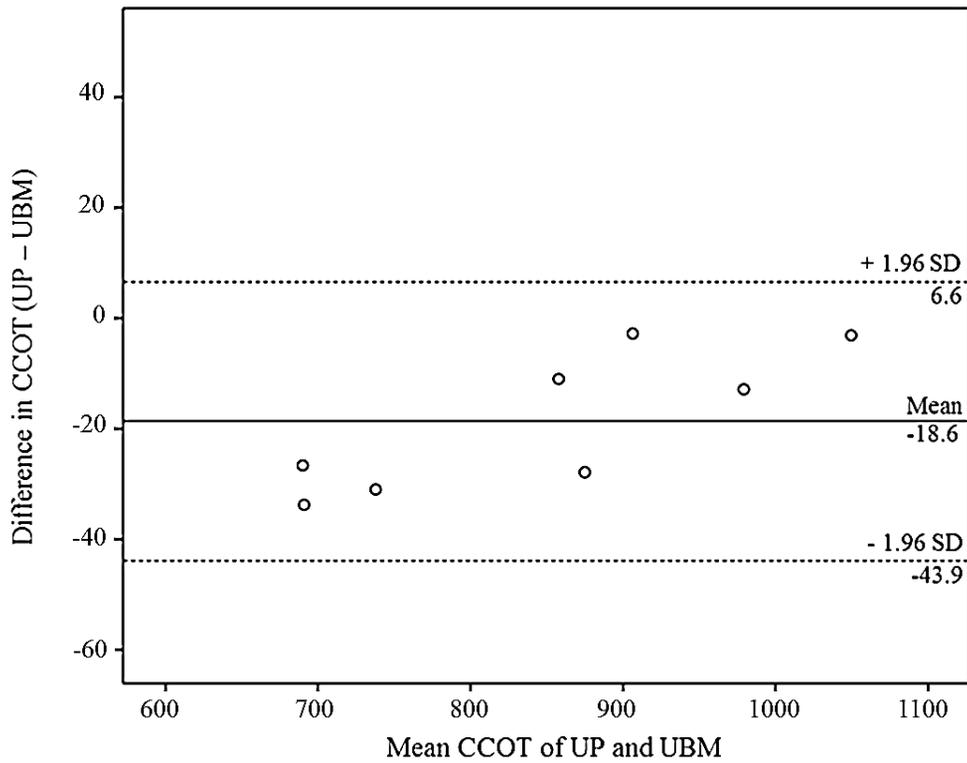


Fig. 4. Bland-Altman plot demonstrating the mean of central corneal thickness (CCOT) values between ultrasound pachymetry (UP) and ultrasound biomicroscope (UBM). Mean difference was represented by the middle solid line, and the upper and lower 95% limits of agreement (LoA) were represented by the dashed lines on the side.

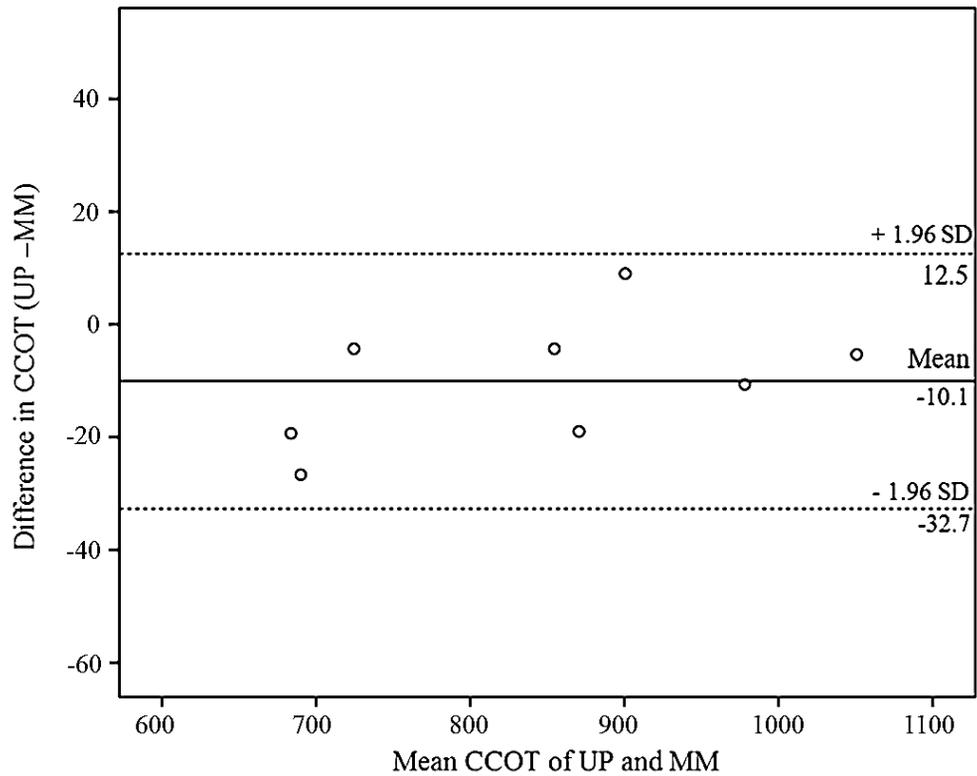


Fig. 5. Bland-Altman plot demonstrating the mean of central corneal thickness (CCOT) values between ultrasound pachymetry (UP) and manual measurement (MM). Mean difference was represented by the middle solid line, and the upper and lower 95% limits of agreement (LoA) were represented by the dashed lines on the side.

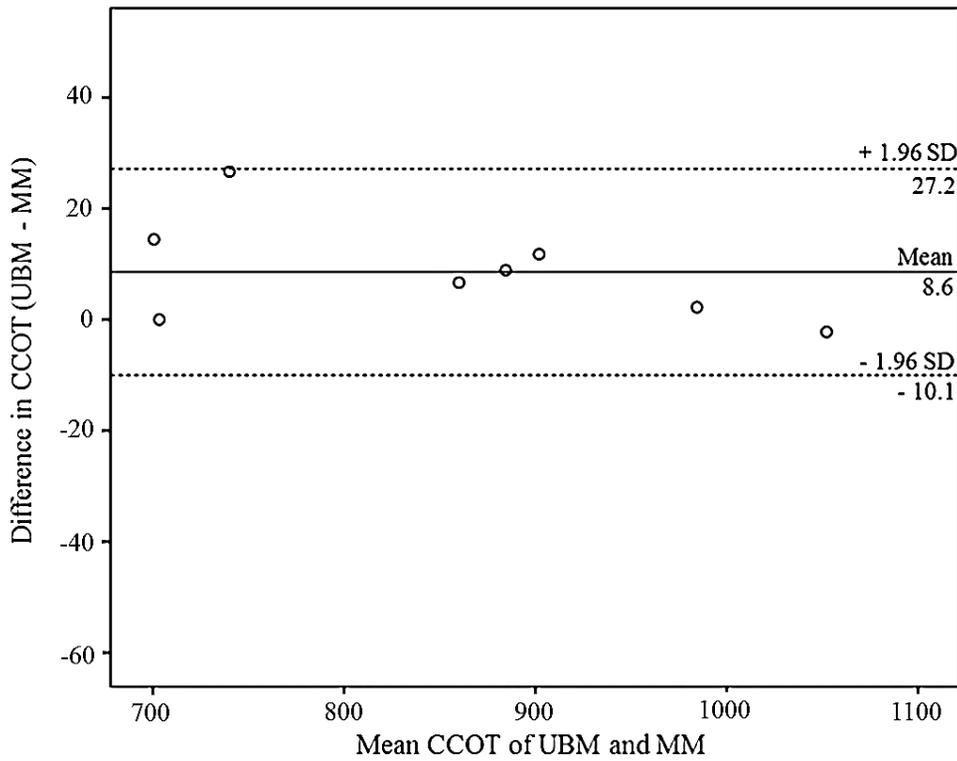


Fig. 6. Bland-Altman plot demonstrating the mean of central corneal thickness (CCOT) values between ultrasound biomicroscope (UBM) and manual measurement (MM). Mean difference was represented by the middle solid line, and the upper and lower 95% limits of agreement (LoA) were represented by the dashed lines on the side.

Discussion

This study compared CCOT measurements by UP, UBM, and MM to assess the accuracy of the devices on frozen canine corneas. In two canine studies, the value of CCOT measured by UP was 589.7 ± 32.3 , and 555.5 ± 17.2 μm on normal canine corneas (Alario and Pirie, 2014; Strom *et al.*, 2016). These values were about 250 μm thinner than CCOT values obtained from frozen corneas in this study, suggesting that the frozen cornea was thicker than the normal cornea due to corneal edema.

In the case of corneal perforation or descemetocoele in dogs, frozen lamellar corneal grafting or full-thickness grafting is an effective treatment for preserving optical function. These techniques and postoperative care have progressively developed over the years (Hansen and Guandalini, 1999; Lacerda *et al.*, 2016). The COT of the donor graft is recommended to 70-80% thickness of recipients and accurate measurement of COT is also necessary to determine corneal changes from graft rejection (Lacerda *et al.*, 2016). Therefore, it is important to evaluate whether the devices operate normally in edematous corneas.

In some previous reports, COT measured at a peripheral location was compared with that at a central location. In both humans and dogs, the PCOT was significantly thicker on average than CCOT (Gilger *et al.*, 1991; González-Méijome *et al.*, 2003; Haque *et al.*, 2008; Strom *et al.*, 2016). In contrast to previous studies, the present study demonstrated that the difference between CCOT and PCOT was statistically insignificant in all devices. It demonstrated that the change of CCOT was greater than that of PCOT in frozen eyes. This was because the central cornea was more

vulnerable to corneal edema than the peripheral cornea. In human, it was reported that the corneal edema was present 20-50% more in the central than in the peripheral cornea (Bonanno and Polse, 1985). In veterinary ophthalmology, the result was reported to be similar to that of humans. The increased central corneal edema was probably caused by distinction of structure or hydration characteristics between central and peripheral cornea (Ling, 1987). The edematous cornea itself might also be another reason for the insignificant difference between CCOT and PCOT. It was reported that the reliability of values obtained by ultrasound devices was dependent on corneal thickness. It was indicated that the measurements of thick COT were less reliable than those of the thin COT (González-Méijome *et al.*, 2003; Strom *et al.*, 2016).

Intraobserver repeatability of COT measurements within each device was represented by the variability of repeated measurements. The ICC values for measurements within each device was highly significant. This was indicated excellent reproducibility and reliability for each device.

Based on difference of upper and lower 95% LoA between UP and UBM, between UP and MM, and between UBM and MM, the change in CCOT was represented 5.9%, 5.3%, and 4.3%, respectively. In dogs, the mean diurnal pachymetric variation of CCOT during the 12-hour period was recorded as 7.5% (Martín-Suárez *et al.*, 2014). The differences of measurements between UP and UBM, between UP and MM, and between UBM and MM were all smaller to that of the diurnal variation. This was indicated that a high level of agreement among devices was shown, the

difference of measurements was clinically acceptable, and the three devices could be used interchangeably (Hashemi *et al.*, 2009).

Although there were no significant differences among the three methods in this study, there was a previous study in which the value of CCOT measured with UP was less than that measured with UBM (Tam and Rootman, 2003). There was also a previous study showing the opposite result (Al-Farhan and Al-Otaibi, 2012). As compared to other devices for measuring corneal thickness as well as comparison between the two devices, there were severe conflicting results about values obtained using UP (Bechmann *et al.*, 2001; González-Méijome *et al.*, 2003; Al-Mezaine *et al.*, 2008; Ishibazawa *et al.*, 2011; Alario and Pirie, 2014; Strom *et al.*, 2016). Because the probe of UP must be manually placed in direct contact with the cornea, the variation of measurements with UP depends on the competence of users (Correa-Perez *et al.*, 2012). Not only perpendicular placement of the probe in the center of the cornea, but also delicate probing for minimizing the risk of corneal damage is required in UP (Miglior *et al.*, 2004). In addition, the use of ultrasound probe can result in applanation force, pushing away easily the precorneal tear film and the corneal epithelium, thus making the COT thin by compression (Nissen *et al.*, 1991; Pierro *et al.*, 1998; Tam and Rootman, 2003). Although measurements of UP have variability, the UP has been a common, accessible, and convenient method to measure CCOT in veterinary ophthalmology (Al-Farhan and Al-Otaibi, 2012; Alario and Pirie, 2014). The device also has the advantage of working well in pathological corneas such as pigmented corneas (Alario and Pirie, 2014).

In veterinary ophthalmology, UBM has been recognized as a noninvasive device to acquire high resolution images of the anterior segments of the globe (Bentley *et al.*, 2005). However, it is controversial whether UBM can be used as a device for measuring the thickness of the cornea. Some studies have documented that the measurement of CCOT using the UBM was not statistically different in comparison to UP and therefore UBM was also a reliable alternative to UP for measurement of CCOT in normal corneas (Pierro *et al.*, 1998; Tam and Rootman, 2003). In contrast, other studies have reported that the accuracy of UBM for measuring CCOT was unreliable, and that is not an appropriate tool to measure corneal thickness (Urbak, 1999; Al-Farhan and Al-Otaibi, 2012). The difference in results among previous studies depended on the skill of the operator and was affected by subjective interpretation of visualized images (Urbak *et al.*, 1998). Although UBM required operating skills and a longer procedure time to obtain high quality images to maximize centrality and perpendicularity, it provided the benefit of visualization, compared with UP. The end of the UBM probe was also soft with transduction fluid being immersed. Because the hard part of the probe was not in direct contact with the cornea, corneal epithelium and precorneal tear film was less influenced than with the UP (Pierro *et al.*, 1998; Tam and Rootman, 2003; Al-Farhan and Al-Otaibi, 2012).

Direct measurement of COT was almost impossible without anatomical and physiological damage to the cornea. Therefore, studies of true COT values have been developed to compare different measuring devices. UP has been used as the gold standard for measuring COT since it is difficult to examine a value of true COT *in vivo*. In this study, the COT was measured directly using a digital caliper. The value

measured by this method might be the closest to the actual corneal thickness. A previous study reported that the variability of measurements using the digital calipers was consistent across users and commercially available digital calipers in ophthalmic measurements were precise and reliable (Mohamed *et al.*, 2013).

There were severe limitations in this study. Firstly, there was a relatively small sample size. Although all tests for normality in each group were satisfied and the results of comparison were statically significant, a larger sample size would have regulated the distribution of values, reduced the variance, and increased confidence in the statistics. At second, the measurements were performed in a single breed population. All beagle dogs in this study were male and relatively young. In dogs, COT was thicker in males than in females and was reported to become thicker with an increase in age and body weight (Gilger *et al.*, 1991; Rüfer *et al.*, 2007). Further studies would be needed to elucidate the effects of age, sex and body weight.

Conclusions

The measurements using the UP, UBM, and MM showed statistically similar COT values on edematous frozen corneas in dogs. There was good agreement among the three methods, and the devices demonstrated excellent intraobserver repeatability with clinically acceptable differences. The UBM and UP provided accurate and reliable measurements comparable to MM which was similar to the true COT of frozen corneas. It is considered that both UP and UBM can be used as clinically applicable methods for measuring CCOT in frozen corneas of dogs.

References

- Al-Farhan HM, Al-Otaibi WM, 2012. Comparison of central corneal thickness measurements using ultrasound pachymetry, ultrasound biomicroscopy, and the Artemis-2 VHF scanner in normal eyes. *Clinical Ophthalmology* 6, 1037-1043.
- Al-Mezaine HS, Al-Amro SA, Kangave D, Sadaawy A, Wehaib TA, Al-Obeidan S, 2008. Comparison between central corneal thickness measurements by oculus pentacam and ultrasonic pachymetry. *International Ophthalmology* 28, 333-338.
- Alario AF, Pirie CG, 2014. Central corneal thickness measurements in normal dogs: a comparison between ultrasound pachymetry and optical coherence tomography. *Veterinary Ophthalmology* 17, 207-211.
- Bechmann M, Thiel MJ, Neubauer AS, Ullrich S, Ludwig K, Kenyon KR, Ulbig MW, 2001. Central corneal thickness measurement with a retinal optical coherence tomography device versus standard ultrasonic pachymetry. *Cornea* 20, 50-54.
- Bentley E, Miller PE, Diehl KA, 2005. Evaluation of intra-and interobserver reliability and image reproducibility to assess usefulness of high-resolution ultrasonography for measurement of anterior segment structures of canine eyes. *American Journal of Veterinary Research* 66, 1775-1779.
- Bonanno JA, Polse KA, 1985. Central and peripheral corneal swelling accompanying soft lens extended wear. *Optometry and Vision Science* 62, 74-81.

- Bovelle R, Kaufman SC, Thompson HW, Hamano H, 1999. Corneal thickness measurements with the Topcon SP-2000P specular microscope and an ultrasound pachymeter. *Archives of Ophthalmology* 117, 868-870.
- Correa-Perez ME, López-Miguel A, Miranda-Anta S, Iglesias-Cortinas D, Alió JL, Maldonado MJ, 2012. Precision of high definition spectral-domain optical coherence tomography for measuring central corneal thickness. *Investigative Ophthalmology and Visual science* 53, 1752-1757.
- Dada T, Sihota R, Gadia R, Aggarwal A, Mandal S, Gupta V, 2007. Comparison of anterior segment optical coherence tomography and ultrasound biomicroscopy for assessment of the anterior segment. *Journal of Cataract and Refractive Surgery* 33, 837-840.
- Gilger B, Whitley R, McLaughlin S, Wright J, Drane J, 1991. Canine corneal thickness measured by ultrasonic pachymetry. *American Journal of Veterinary Research* 52, 1570-1572.
- Gilger B, Wright J, Whitley R, McLaughlin S, 1993. Corneal thickness measured by ultrasonic pachymetry in cats. *American Journal of Veterinary Research* 54, 228-230.
- González-Méijome JM, Cerviño A, Yebra-Pimentel E, Parafita MA, 2003. Central and peripheral corneal thickness measurement with Orbscan II and topographical ultrasound pachymetry. *Journal of Cataract and Refractive Surgery* 29, 125-132.
- Hansen PA, Guandalini A, 1999. A retrospective study of 30 cases of frozen lamellar corneal graft in dogs and cats. *Veterinary Ophthalmology* 2, 233-241.

- Haque S, Jones L, Simpson T, 2008. Thickness mapping of the cornea and epithelium using optical coherence tomography. *Optometry and Vision Science* 85, 963-976.
- Hashemi H, Yazdani K, Mehravaran S, KhabazKhoob M, Mohammad K, Parsafar H, Fotouhi A, 2009. Corneal thickness in a population-based, cross-sectional study: the Tehran eye study. *Cornea* 28, 395-400.
- Ishibazawa A, Igarashi S, Hanada K, Nagaoka T, Ishiko S, Ito H, Yoshida A, 2011. Central corneal thickness measurements with Fourier-domain optical coherence tomography versus ultrasonic pachymetry and rotating Scheimpflug camera. *Cornea* 30, 615-619.
- Lacerda RP, Peña Gimenez MT, Laguna F, Costa D, Ríos J, Leiva M, 2016. Corneal grafting for the treatment of full thickness corneal defects in dogs: a review of 50 cases. *Veterinary Ophthalmology*, doi: 10.1111/vop.121392 [Epub ahead of print]
- Ling T, 1987. Osmotically induced central and peripheral corneal swelling in the cat. *Optometry and Vision Science* 64, 674-677.
- Martín-Suárez E, Molleda C, Tardón R, Galán A, Gallardo J, Molleda J, 2014. Diurnal variations of central corneal thickness and intraocular pressure in dogs from 8: 00 am to 8: 00 pm. *Canadian Veterinary Journal* 55, 361.
- Miglior S, Albe E, Guareschi M, Mandelli G, Gomasasca S, Orzalesi N, 2004. Intraobserver and interobserver reproducibility in the evaluation of ultrasonic pachymetry measurements of central corneal thickness. *British Journal of Ophthalmology* 88, 174-177.

- Mohamed A, Nankivil D, Pesala V, Taneja M, 2013. The precision of ophthalmic biometry using calipers. *Canadian Journal of Ophthalmology* 48, 506-511.
- Nissen J, Hjortdal J, Ehlers N, Frost K, Sørensen T, 1991. A clinical comparison of optical and ultrasonic pachometry. *Acta Ophthalmologica* 69, 659-663.
- Park YW, Jeong MB, Kim TH, Ahn JS, Ahn JT, Park S, Kim SE, Seo KM, 2011. Effect of central corneal thickness on intraocular pressure with the rebound tonometer and the applanation tonometer in normal dogs. *Veterinary Ophthalmology* 14, 169-173.
- Pierro L, Conforto E, Giordano Resti A, Lattanzio R, 1998. High-frequency ultrasound biomicroscopy versus ultrasound and optical pachymetry for the measurement of corneal thickness. *Ophthalmologica* 212, 1-3.
- Rüfer F, Schröder A, Bader C, Erb C, 2007. Age-related changes in central and peripheral corneal thickness: determination of normal values with the Orbscan II topography system. *Cornea* 26, 1-5.
- Strom AR, Cortés DE, Rasmussen CA, Thomasy SM, McIntyre K, Lee SF, Kass PH, Mannis MJ, Murphy CJ, 2016. In vivo evaluation of the cornea and conjunctiva of the normal laboratory beagle using time and Fourier-domain optical coherence tomography and ultrasound pachymetry. *Veterinary Ophthalmology* 19, 50-56.
- Tam ES, Rootman DS, 2003. Comparison of central corneal thickness measurements by specular microscopy, ultrasound pachymetry, and ultrasound biomicroscopy. *Journal of Cataract and Refractive Surgery* 29, 1179-1184.

- Urbak SF, 1998. Ultrasound biomicroscopy. I. Precision of measurements. *Acta Ophthalmologica* 76, 447-455.
- Urbak SF, Pedersen JK, Thorsen T, 1998. Ultrasound biomicroscopy. II. Intraobserver and interobserver reproducibility of measurements. *Acta Ophthalmologica* 76, 546-549.
- Urbak SF, 1999. Ultrasound biomicroscopy. III. Accuracy and agreement of measurements. *Acta Ophthalmologica* 77, 293-297.

국 문 초 록

개의 냉동 각막에서 초음파각막두께측정계, 초음파생체현미경과 디지털캘리퍼를 이용한 각막 두께 측정법의 비교

지도교수 서 강 문

정 서 우

서울대학교 대학원

수의학과 임상수의학 전공

본 연구는 개의 냉동 각막에서 초음파각막두께측정계 (ultrasound pachymetry), 초음파생체현미경 (ultrasound biomicroscopy) 과 디지털캘리퍼를 이용한 직접 측정방법을 이용하여 각막 두께를 측정하고 그 값을 비교함으로써 각 측정법들의 임상적 효용성을 평가하고자 실시하였다.

-20℃에서 4주간 보관했던 총 8개의 개의 냉동 안구를 실험에 사용하였으며, 세 가지 방법으로 동일한 5개의 지점에서 각 각막의 두께를 측정하였다(중심, 3시, 6시, 9시와 12시 방향). 세 가지 측정법에 따른 결과를 비교분석하였고, 모든 값들은 동일한 측정자에 의해 세 번씩 측정되어 그 평균값을 사용하였다.

초음파각막두께측정계, 초음파생체현미경, 디지털캘리퍼를 이용한 직접 측정법으로 측정된 결과를 분석하였을 때, 평균 중심 각막 두께는 각각 $839.0 \pm 138.2 \mu\text{m}$, $857.6 \pm 127.9 \mu\text{m}$ 그리고 $849.1 \pm 132.8 \mu\text{m}$ 였고, 세 측정값은 각각 통계적으로 유의적인 차이를 보이지 않았다 ($P > 0.05$). 각 측정법 사이의 일치도를 보기 위한 Bland-Altman plots상에서 세 측정법은 높은 일치도를 보였다. 또한 주변부 각막에서도 세 측정법 비교에 있어서 중심 각막과 통계적으로 비슷한 결과를 나타내었으며, 중심 각막 두께와 주변부 각막 두께의 측정값 사이에서도 통계적으로 유의적인 차이를 보이지 않았다.

본 연구 결과 개의 냉동안에서 초음파 각막두께측정계와 초음파 생체현미경을 이용한 각막 두께의 측정은 직접 측정법에 의한 측정과 유의적인 차이를 보이지 않음이 확인되었다. 따라서 초음파각막두께측정계와 초음파생체현미경은 각막 두께 측정을 위해 사용할 수 있는 정확하고 신뢰할 수 있는 방법으로 판단된다.

주요어: 각막 두께, 초음파각막두께측정계, 초음파생체현미경, 냉동 각막, 개

학번: 2015-23165