

COMPARISON OF FOUR METHODS FOR CORNEAL THICKNESS MEASUREMENT

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Summary

The aim of the study was to evaluate the thickness of the normal cornea in order to establish correlation between four methods of measuring including: ultrasound pachymetry (USP), anterior segment optical coherence tomography (ASOCT), non-contact tonometry (TONOPACHY) and laser-scanning confocal microscopy (LSCM). The study was based on evaluating repeatability and comparability of four different methods for measuring the corneal thickness. Non contact specular microscopy was first performed on all 27 patients (aged between 20 and 24 years) to evaluate corneal characteristics and confirm the absence of pathological changes. Each participant was examined by USP, ASOCT and TONOPACHY, and 13 eyes of 10 persons were also examined by LSCM. The values of average central corneal thickness measured by USP, ASOCT, TONOPACHY and LSCM were $532.20 \pm 4.5 \mu\text{m}$, $553.33 \pm 12.1 \mu\text{m}$, $548.20 \pm 5.62 \mu\text{m}$ and $573.33 \pm 7.22 \mu\text{m}$, respectively. There was a high correlation between the instruments. The mean differences for central corneal thickness measurements were $41.43 \pm 1.67 \mu\text{m}$ between USP and LSCM, $20.43 \pm 2.4 \mu\text{m}$ between USP and ASOCT, and $22.1 \pm 3.88 \mu\text{m}$ between USB and TONOPACHY. Anterior segment optical coherence tomography overestimated corneal thickness as compared with that measured by USP, which is believed to be a gold standard. Anterior segment optical coherence tomography had better agreement with USP, as compared with LSCM. However, the results of measured cornea thickness by TONOPACHY were very close to cornea thickness measured by ASOCT.

Key words: cornea thickness, pachymetry, optical coherence tomography, confocal microscopy

Introduction

The measurement of central corneal thickness has become increasingly important in ophthalmic practice[1]. Central corneal thickness (CCT) measurement is an essential part of diagnostic and therapeutic approaches to corneal pathology. Corneal thickness measurement method should be repeatable and reproducible to be accepted as an ideal method. This method should be preferably non-contact and safe, as well as quick and easy to perform. The measurement of corneal thickness has

various important applications. Monitoring central corneal thickness is important for diagnosing and standing of ectatic dystrophies such as keratoconus, and calculating the risk of glaucoma progression in ocular hypertension patients [2, 3]. It is also essential for preoperative risk selection and planning of refractive surgical procedures.

Traditional methods of corneal thickness measurement use spot pachymetry techniques. Ultrasound pachymeter is one such method. It is a method both cheap and easy to use for corneal measurement [4]. Many other instruments can assess CCT, e.g. ultrasound pachymetry (USP) or ultrasound biomicroscopy (UBM), specular and confocal microscopy, slip-scanning corneal topography, the Scheimpflug system, optical biometry, and optical coherence tomography (OCT). *In vivo* laser scanning confocal microscopy (LSCM) is a new non-invasive technology, which is useful as a supplementary diagnostic tool for *in vivo* assessment of the histopathology of many ocular surface disorders. This technology allows observation of all cornea levels. Ultrasound pachymetry used to be a gold standard for measuring corneal thickness. It is a contact method and the result of measurement strongly depends on the operator technique [5]. The precision of measurements is estimated by two terms: reproducibility and repeatability. Reproducibility is the variability of results obtained from one object using the same device in different measurement conditions. The variability of results obtained from one object in the same measurement conditions (time, instrument, technique, place, and operator) defines repeatability [6].

The purpose of this study was to evaluate the thickness of normal cornea in order to establish correlation between four methods of measuring including: ultrasound pachymetry (USP), anterior segment optical coherence tomography (ASOCT), non-contact tono/pachymetry (TONOPACHY) and Laser-scanning confocal microscopy (LSCM).

Materials and Methods:

The study was based on evaluating repeatability and comparability of four different methods for measuring the corneal thickness. Non contact specular microscopy was first performed on all 27 adults to evaluate corneal characteristics and confirm that there were no pathological changes.

The mean age of the study group patients was 21.2 ± 1.35 years (range 20-24 years). The group included 23 female (85.2%) and 4 male (14.8%) patients. Each patient was examined by ultrasound pachymetry (USP), anterior segment optical coherence tomography (ASOCT), non-contact tono/pachymetry (TONOPACHY). Ten persons with 13 eyes were examined by Laser-scanning confocal microscopy (LSCM) and the other three methods.

In vivo laser scanning confocal microscopy examination

In vivo confocal microscopy enables detailed analysis of the human cornea, allowing detailed visualization of the corneal microstructure. The LSCM uses a coherent high intensity light source and the laser beam is scanned over the back of the microscope objective by a set of galvanometer scanning mirrors. In this study we used Heidelberg Retina Tomography/Rostock Cornea Module (Heidelberg Engineering GmbH, Germany), which is an applanating device. A 63x water immersion objective lens (Zeiss) is used with a 670 nm wavelength Class I diode laser as a light source to allow a scanning area of $300 \times 300 \mu\text{m}$ (FOV- $300 \mu\text{m}$), with a lateral resolution of $1 \mu\text{m}/\text{pixel}$ and z-resolution of $4 \mu\text{m}$ and up to 800x magnification. The objective of the microscope was covered with a disposable sterile PMMA cap previously filled with eye gel (Corneregel) in order to provide immersion. One drop of topical anesthetic (Alcain 0, 5%) was applied in the inferior cul-de-sac of the eye to be examined. The patient was positioned in front of the instrument, his/her chin and forehead adjusted against the headrests. The instrument was justified using the inbuilt software. The objective of the microscope was subsequently advanced to achieve a contact with the ocular surface; the position of the eye was monitored by a camera placed on the side of the objective.

Two hours after *in vivo* confocal microscopy other measurements were performed in the following order:

Optical coherent tomography examination

Optical coherent tomography was performed using 3D Topcon 2000 OCT with lateral resolution of $\leq 20 \mu\text{m}$ and in-depth resolution of $5 \mu\text{m}$ - $6 \mu\text{m}$. Optical coherence tomography (OCT) is a non-contact imaging method based on principles of low-coherence interferometry. Operating distance for anterior segment (AS) photography was 63,7 mm (with anterior

segment special forehead rest); scan speed was 27 000 A-scans per second; scanning range on cornea 6x6mm. A standardized scanning protocol was performed for each patient. High resolution anterior segment OCT scans 12 radial (1024 A line) and 3D (6x6mm, 512x128) were carried out. All exams were performed with centering on the visual axis. A longer wavelength light source is used in anterior segment (AS)-OCT. That imaging technique allows higher resolution imaging of the anterior and posterior cornea surfaces. This new generation high-speed scanning system exceeds the generation of pachymetric maps [7, 8].

Tono/pachymetry examination

The Non-Contact Tonometer/Pachymeter is the integrated pachymeter/tonometer, combining the measurement of intraocular pressure (IOP) and CCT in one instrument. This IOP measurement is easy, quick and accurate. Non-contact pachymetry is a reliable method, easy to use and without subjective factor of the operator measurement skills. The tono/pachymetry was designed in response to numerous clinical studies. All these studies have shown the importance of simultaneous measurement of central corneal thickness and intraocular pressure. Tono/pachymetry readings were taken first to avoid any potential disturbance caused by

corneal contact in the ultrasonic pachymetry. For examination the patient was positioned with the chin in a cup and forehead against a headband. The CCT was measured while the subject focused on a fixation light in the instrument. After proper positioning of the alignment dot, circle, and bar on the screen, pachymetry was performed [9].

Ultrasound Pachymetry examination

Ultrasound Pachymetry thickness was executed using an A-scan ultrasonic pachymetry. This instrument was precalibrated for all measurements. Alcain (0.5% collyr, Alcon) was used for topical anesthesia. All the patients were seated upright and the handheld probe of ultrasound pachymetry examination was aligned as perpendicularly as possible on the central cornea. Twelve readings were obtained and averaged.

Results

The group included 23 female (85.2%) and 4 male (14.8%) patients. The mean age of the study group was 21.2 ± 1.35 years (range, 20-24 years).

The results of Mean Central Corneal Thickness are shown in Table 1.

Table 1. Mean central corneal thickness values in all 27 examined eyes in healthy patients with four different methods

Method of Measurement	Mean Central Corneal Thickness ($\mu\text{m} \pm \text{SD}$)
Ultrasound pachymetry (USP)	$532.20 \pm 4.5 \mu\text{m}$
Anterior segment optical coherence tomography (ASOCT)	$553.33 \pm 12.1 \mu\text{m}$
Non contact tono/pachymetry (TONOPACHY)	$548.20 \pm 5.62 \mu\text{m}$
Laser-scanning confocal microscopy (LSCM)	$573.33 \pm 7.22 \mu\text{m}$

There was high correlation between instruments. The CCT measurements were significantly deeper with the LSCM than other three methods. The lowest value of CCT was measured by USP. The mean differences for central corneal thickness measurements were $41.43 \pm 1.67 \mu\text{m}$ between USP and LSCM, $20.43 \pm 2.4 \mu\text{m}$ between USP and ASOCT, and $22.1 \pm 3.88 \mu\text{m}$ between USB and TONOPACHY. Table 2 shows the interdevice differences. The results of measured cornea thickness by TONOPACHY are very close to cornea thickness

measured by ASOCT. The biggest alternation between the given results is this measured by LSCM. LSCM overestimates with more than $40 \mu\text{m}$ the USP mean value.

ASOCT had better agreement with USP, as compared with LSCM. The results of LSCM measurements shown the biggest values and overestimated corneal thickness compared with that measured with USP. In the same time ASOCT overestimated corneal thickness compared with that measured with USP, which is believed to be a gold standard.

Table 2. Interdevice difference between CCT measurements

Devices	Difference
USP / LSCM	41.43±1.67 μ m
USP / AS-OCT	20.43±2.4 μ m
USP / Tono-Pachymetry	22.1±3.88 μ m

Discussion

Accurate measurement of CCT has become important with the rapidly increasing popularity of corneal refractive surgical procedures and with the implantation of new (phakic) intraocular lenses. In this study, we have compared four different: two noncontact and two contact optical methods of measuring CCT. Three of these instruments, the LSCM, the ASOCT and the non-contact tonopachymetry have been made commercially available only quite recently. The clinical importance of corneal thickness measurement is increasing. It is useful for diagnosing and monitoring. The value of intraocular pressure is in correlation with corneal thickness and the thickness of the cornea is important for calculating glaucoma progression risk in patients with ocular hypertension. Underestimation of intraocular pressure may lead to thinner corneas [10-14].

The ultrasound pachymetry (USP) has been considered a gold standard for corneal thickness measurement for many years [15-17]. It is a contact method. However, many other methods, including AS-OCT and TONOPACHY have the great advantage of noncontact. These methods use different measurement technologies. Their results are also different. According to different researchers, CCT measurement obtained with standard ultrasound pachymetry varies from 542 to 550 μ m [17-20]. In our case, the mean CCT measured by the non-contact pachymetry was 548.20±5.62 μ m. This result is very close to the result of CCT by ASOCT. Our result of ASOCT measurements was 548.20±5.62 μ m. In the literature, the mean CCT previously reported was within the range of 523-527 μ m. The CCT was measured by different types of OCT instruments [21, 22].

We decided to use US pachymetry as the reference for CCT measurement in our study because new topography devices are calibrated against the US pachymeter. However, as Bourne and McLaren [22] point out, the most accurate

pachymetry measurements can be obtained with optical pachymeters, such as the one described by Maurice and Giardini [23]. Nevertheless, ultrasonic pachymeters are fast, simple to use, and widely available, and they provide repeatable measurements, explaining why many recent studies use this method as a reference [24-26].

Piotrowski reported mean results of CCT measured by AS-OCT was lower than results from USP [9]. These results correspond with other studies with Pentacam HR and ASOCT measurements. The results were lower than ultrasound pachymetry measurements. There is a hypothesis that the topical anesthetics used in contact measurement with the ultrasound pachymeter may cause corneal epithelial edema and overestimation of the values of the CCT [27].

However, there are also studies [9, 28] showing just the opposite correlation. Results from ultrasound pachymetry are lower in comparison to other methods. The ultrasound pachymetry causes tear film dislocation and epithelium compression. CCT measurements are lower by 7-30 μ m.

Wong and coauthors [29] compared central corneal thickness measurements of Orbscan (also with an acoustic factor of 0.92), USP, and OCT. The measured central corneal thickness was similar for Orbscan (555.96±32.41 μ m) and USP (555.11±35.30 μ m) and thinner for OCT (523.21±33.54 μ m).

In vivo laser scanning confocal microscopy (LSCM) is a new non-invasive technology useful as a supplementary diagnostic tool for *in vivo* assessment of the normal structure or histopathology of the cornea. Jalbert describe *in vivo* laser scanning examination principle of normal cornea examination. Confocal microscopes can be used to measure corneal thickness [29-33]. This function is called confocal microscopy through focusing (CMTF) on the TSCM (tandem scanning confocal microscope) [30]. Briefly, rapid movement of the objective lens itself [31] or of the focus of the objective lens [30] in the Z-axis is automated and registered by a computer. The amount of light backscattered by the central section of each image is also recorded, allowing the generation of an intensity profile curve. Specialized software allows interactive viewing of the image corresponding to the cursor location on the Z-curve and measurement of the distance between any two points on the curve. Because different layers of the cornea reflect light at different intensities, the depth-intensity profile allows for

the determination of corneal sub layer location. As well as corneal thickness, measurements of epithelial thickness, Bowman's layer thickness, and following laser in situ keratomileusis (LASIK) surgery, flap thickness can also be obtained[30, 32].

In this study, we measured only normal corneas of healthy subjects. Therefore, we do not have data concerning the agreement between the four methods when measuring corneas with pathological alterations, or postoperative corneas.

Conclusion

In conclusion, we have shown that ASOCT overestimated corneal thickness compared with that measured with USP, which is believed to be a gold standard. Also ASOCT had better agreement with USP, as compared with LSCM. However the results of measured cornea thickness by TONOPACHY are very close to cornea thickness measured by ASOCT.

Further research is needed to estimate central corneal thickness measurements by these technologies and come to a conclusion whether USP results are more or less clinically useful than others.

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