ACURACY OF CENTRAL AND PERIPHERAL CORNEA THICKNESS: A COMPARISON OF TWO CLINICALLY AVAILABLE INSTRUMENTS

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ABSTRACT

INTRODUCTION: To compare the thickness measurements of central and peripheral cornea in healthy human subjects without eye symptomology, using laser scanning confocal microscopy (LSCM) and anterior segment OCT (AS-OCT), two technologies based on optical principle.

MATERIALS AND METHODS: The study was based on evaluating repeatability and comparability of two different methods for measuring the corneal thickness in central and four peripheral corneal zones. Data were analyzed in two age groups, group A, 22±2 years (n= 27) and group B, 60±11 years (n=29). Fifty six subjects were clinically examined to observe corneal structure and confirm that there are no pathological changes. Each participant was then examined by AS-OCT and following topical anaesthesia by LSCM.

RESULTS: The mean values of average central corneal thickness **by anterior segment optical coherence tomography (AS-OCT) and Laser-scanning confocal microscopy (LSCM) in the group of younger individuals were 553.33±12.1 μm and 573.33±57.22 μm, respectively for the central cornea and 734.33±22.1 μm and 773.83±72.22 μm for corneal periphery. The group of aged participants presented results of CCT measured by AS-OCT and LSCM as follow: 561.12±19.1 μm and 593.93±60.72 μm, respectively for the central cornea and 742.33±30.1 μm and 789.31±78.92 μm for corneal periphery. LSCM demonstrated lower repeatability.**

CONCLUSION: The study demonstrated that AS-OCT is better method for measurement of corneal thickness of subjects regardless their age. Furthermore the method is less invasive and provides thickness measurement of 70-80% of the corneal surface. The two methods should be combined to achieve comprehensive structural and morphological analysis of the human cornea in health and disease.

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

INTRODUCTION

The measurement of central corneal thickness has become increasingly important in ophthalmic practice (2). Central corneal thickness (CCT) measurement plays a major role in diagnostic and therapeutic approaches to corneal pathology and has an important value for intraocular pressure readings. An ideal method of corneal thickness measurement should be accurate, repeatable, reproducible, and safe (preferably non-invasive), as well as easy and quick to perform at low cost. The measurement of corneal thickness has various important applications in monitoring corneal edema and ectatic dystrophies such as keratoconus, measuring intraocular pressure, and calculating risk of progression to glaucoma in patients with ocular hypertension (7,10). It is also

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essential to the selection of patients for and the planning of refractive surgical procedures.

Traditional methods of corneal thickness measurement employ spot pachymetry techniques, such as ultrasound pachymeters (USP), which are reliable, easy to use, and inexpensive (30). CCT can be assessed by means of many instruments, including specular microscopy, confocal microscopy, ultrasound pachymetry, ultrasound biomicroscopy, slit-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 useful as a supplementary diagnostic tool for in vivo assessment of the histopathology of many ocular surface disorders. With this technology one can observe the cornea levels.

The precision of measurements can be measured by repeatability and reproducibility. Repeatability is defined as the variability of results obtained from one object in the same measurement conditions (time, instrument, technique, place, and operator), while reproducibility is the variability of results obtained from one object using the same device in different measurement conditions (24,27).

The purpose of this study was to analyze the thickness of the normal cornea in order to evaluate the thickness measurements of central and peripheral cornea thickness in healthy humans without eye symptomology using laser scanning confocal microscopy (LSCM) and anterior segment OCT (AS-OCT).

MATERIALS AND METHODS

The study was based on evaluating repeatability and comparability of two different methods for measuring the corneal thickness in central and four peripheral zones. Data were analyzed in two age groups: group A, 22±2 years (n= 27) and group B, 60±11 years (n=29). All fifty six subjects were clinically examined to observe corneal structure and confirm that there are no pathological changes. Each participant was then examined by AS-OCT and following topical anaesthesia by LSCM.

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 300x300 μm (FOV-300 μm), with a lateral resolution of 1 μm/pixel and z-resolution of 4 μm 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.

Optical coherent tomography examination:

Optical coherent tomography was performed using 3D Topcon 2000 OCT with lateral resolution of ≤20μm and in-depth resolution of 5μm-6μm. Optical coherence tomography (OCT) is a non–contactimaging technique 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. Anterior segment (AS)-OCT employs a longer wavelength light source that allows higher resolution imaging and better delineation of the anterior and posterior surfaces of the cornea with the computer tracing algorithm.6 Its high-speed scanning system

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enables the generation of pachymetric maps, in addition to linear cross-sectional images (7,8).

RESULTS

The data were analyzed in two age groups. First group A included 27 participants. The mean age of the study group A was 22±2 years (range, 20-24 years)

The results of Mean Central Corneal Thickness and Mean Peripheral Corneal Thickness of Group A are shown in Table 1.

Tabl. 1. Mean central and peripheral corneal thickness values in all 27 examined eyes of group A with two different methods

	LSCM	AS - OCT
Mean Central Corneal Thickness ([µm] +/- SD)	573.33± 57.22 µm	553.33± 12.1 µm
Mean Peripheral Corneal Thickness ([µm] +/- SD)	773.83± 72.22 µm	734.33± 22.1 µm

The mean values of average central corneal thickness by Laser-scanning confocal microscopy (LSCM) and anterior segment optical coherence tomography (AS-OCT) and in the group of younger individuals were 573.33±57.22µm and 553.33±12.1µm, respectively for the central cornea and 773.83±72.22µm and 734.33±22.1µm for corneal periphery.

Second group B represented 29 adults with 58 examined eyes. The mean age of this group was 60±11 years (range, 63-54 years). The group of aged participants presented results of CCT measured by LSCM and AS-OCT as follow: 593.93±60.72 µm and 561.12±19.1 µm, respectively for the central cornea and 789.31±78.92 µm and 742.33±30.1 µm for corneal periphery. There was less repeatability for LSCM.

The results of Mean Central Corneal Thickness and Mean Peripheral Corneal Thickness in participants of Group B are shown in Table 2.

Tabl. 2. Mean central and peripheral corneal thickness values in all 29 examined eyes of participants of group B with two different methods

	LSCM	AS - OCT
Mean Central Corneal Thickness ([µm] +/- SD)	593.93± 60.72 µm	561.12± 19.1 µm
Mean Peripheral Corneal Thickness ([µm] +/- SD)	789.31± 78.92 µm	742.33± 30.1 µm

Using AS-OCT we took photo of cornea slide and measure the cornea thickness as it is shown on Figure 1. AS-OCT also presented Corneal Thickness map with CCT values in all point of the cornea. Measuring of the CCT by LSCM measure the distance between first visualzation ot Epithelium (Figure 3) to the end of Endothelium (Figure 4).

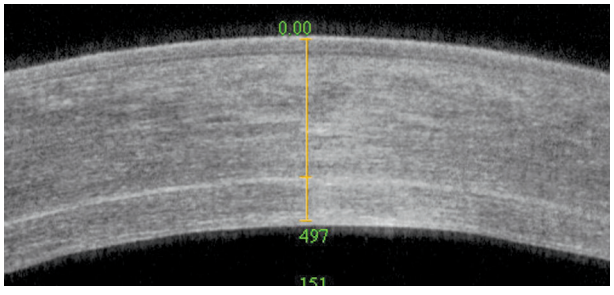

Fig. 1. Slice of cenral cornea and measuring CCT by OCT

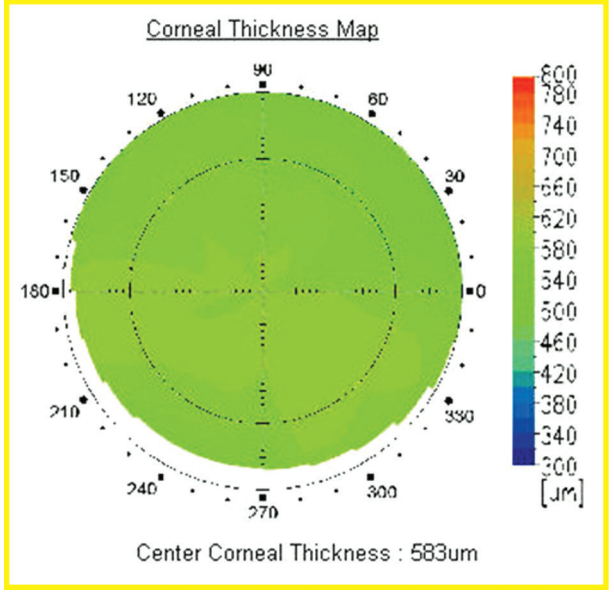

Fig. 2. Central Corneal Thickness map measured by OCT

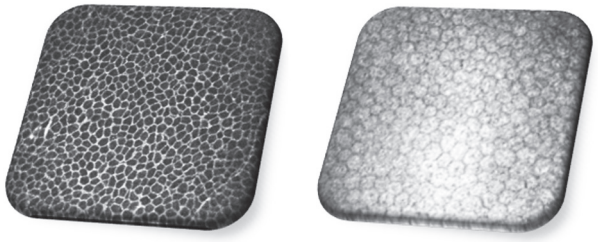

Fig. 3 & Fig. 4. Epithelium and Endothelium of healthy Central Cornea, LSCM

DISCUSSION

In this study, different optical methods of measuring Central Cornea Thickness and Peripheral Cornea Thickness were compared in clinical settings. They are one contact and one noncontact advanced, laser based technologies. These instruments, the LSCM and the AS-OCT have been made commercially available only quite recently.

Corneal thickness measurement is an examination of increasing clinical importance. It is useful for diagnosing and monitoring conditions such as corneal edema. Corneal thickness plays an important role in evaluation of intraocular pressure and glaucoma progression risk in patients with ocular hypertension as thinner corneas may lead to the underestimation of intraocular pressure (7,18,22-23,25).

In vivo laser scanning confocal microscopy (LSCM) is a new non-invasive technology useful as a diagnostic tool for in vivo assessment of the normal structure, or real-time histopathology of the cornea. Jalbert et al. described in vivo laser scanning examination principle of normal cornea examination. Confocal microscopes can be used to measure corneal thickness (15-16,20,29,31). This function is called confocal microscopy through focusing (CMTF) on the TSCM (tandem scanning confocal microscope) (15-16,20,26). Briefly, rapid movement of the objective lens itself (16) or of the focus of the objective lens (15) 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 (15,16).

Lili Ge et al. reported The Role of Axial Resolution of Optical Coherence Tomography on the Measurement of Corneal and Epithelial Thicknesses. The mean CCTs, which they were measured during the first visit using the ultra high resolution OCT, ultra-long scan depth OCT was respectively 529.4±32.0 μm and 527.4±32.4 μm (21). These results are lower than the results of CCT and Peripheral Cornea Thickness in our study.

The peripheral cornea thickness is less reported. In the literature there are limited number of case reports describing peripheral cornea thickness especially using in vivo confocal microscopy, and specifically HRT II Rostock corneal reported.

Usama et al. published study of CCT and Peripheral Cornea Thickness by AS-OCT. They identified that the thinnest point of the cornea as measured from the corneal apex was located predominantly inferotemporal (28). This agreed with results reported by Khoramnia et al. (17), who found that the thinnest location was 92% and 8% in the inferotemporal and superotemporal respectively. Ashwin et al. too reported the thinnest point to be located in the inferotemporal quadrant in more than 96% in right eyes and 81% in left eyes (1). Zheng et al. also noted that the thinnest point located in the inferotemporal quadrant in about 78% of tested eyes (32).

The USP has been considered the gold standard for corneal thickness measurement for many years (5,6,13). However, many other methods can be used, including AS-OCT and Non-Contact Tonometer/Pachymeter, which have the great advantage of noncontact. These methods use different measurement technologies and give different results. According to different researchers, CCT measurement obtained with standard USP varies from 542 to 550μm (4,6,8,9,19). The result is very close to the result of CCT by AS-OCT. Our result of AS-OCT measurements was 573.33±57.22 μm in group A and 593.93±60.72 μm in group B. In literature the mean CCT measured by different types of AS-OCT instruments was previously reported within the range of 523–527 μm (3,14,19).

Piotrowiak reported mean results of CCT measured by AS-OCT was lower than results from USP (12). These results remain in accordance with other studies in which the Pentacam HR and AS-OCT measurements were lower than USP measurements. One possible reason for the difference may be the use of topical anesthetics to take a contact measurement

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with the ultrasound pachymeter which may cause corneal epithelial edema resulting in overestimation of the results (11).

Another research team, Wong and coauthors (31), compared central corneal thickness measurements of Orbscan, USP and AS-OCT. Mean central corneal thickness was similar for Orbscan (555.96±32.41 μm) and USP (555.11±35.30 μm) and thinner for AS-OCT (523.21±33.54 μm).

In the current study we had similar results to the published literature for the OCT, however the newer in vivo confocal technology HRT corneal module, provides less reliable results then tandem scanning technology. The later is with limited use recently, because of the low quality images of the cellular structures of the cornea only. HRT II corneal module is superb from all in vivo confocal technologies, however our study proved that it is not recommended as a corneal tickness measurement tool. Therefore the two technologies OCT and HRTII in vivo confocal microscopy should be combined for better understanding of corneal pathology.

CONCLUSION

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

The laser-scanning confocal microscopy allows ophthalmic clinicians and researchers to visualise living tissues at greatly increased resolutions. Qualitative observations of the images obtained are rapidly giving way to sophisticated quantitative image analysis systems. The good correlation observed between central and peripheral measurements suggests that for most purposes the central corneal thickness can serve as a good guide for predicting peripheral thickness.

The study demonstrated that AS-OCT is better method for measurement of corneal thickness of subjects regardless their age. Furthermore the method is less invasive and with better repeatability. The two methods should be combined for better structural and morphological analysis of the human cornea in health and disease.

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