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ASSESSMENT OF AGREEMENT AMONG THREE MODALITIES FOR CENTRAL CORNEAL THICKNESS MEASUREMENT: ULTRASOUND PACHYMETRY, AS-OCT, AND SPECULAR MICROSCOPY

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ABSTRACT
The cornea plays a critical role in maintaining normal visual function. There are several important clinical parameters such as central corneal thickness (CCT), which is used to diagnose glaucoma, intraocular pressure measurement, refractive surgical planning and other corneal diseases. However, due to variation in measurement results provided by different instruments for determining the thickness of the cornea, it is important to evaluate their correlation and clinical interchangeability. This hospital-based cross-sectional study involved 355 participants above 40 years of age with clinically healthy corneas who visited the Ophthalmology OPD. One-way ANOVA followed by Tukey post hoc test was used for the analysis, where a p-value less than 0.05 was considered as statistically significant. The average age of the study population was 48.64 ± 7.01 years, and there were 56.6% females in the group. Ultrasound Pachymetry always provided the highest average value of CCT (524.66 ± 25.50 µm) followed by AS-OCT (512.54 ± 25.08 µm) and Specular Microscopy (505.35 ± 25.14 µm). Significant differences were noted between all three measurement methods for both eyes (ANOVA, $p < 0.001$). Pairwise comparison revealed that the maximum average difference was between Ultrasound Pachymetry and Specular Microscopy (19.31 µm), while the minimum one was between AS-OCT and Specular Microscopy (7.19 µm), and all differences were statistically significant ($p < 0.001$). Though Ultrasound Pachymetry, AS-OCT and Specular Microscopy showed a reliable measurement of central corneal thickness, there were some significant systematic differences among these three modalities. Ultrasound Pachymetry gave the highest values and is considered the clinical gold standard. AS-OCT and Specular Microscopy are reliable non-contact methods, yet they cannot be used interchangeably with Ultrasound Pachymetry when serial follow-up of a patient is necessary...

KEYWORDS: Central corneal thickness; Ultrasound pachymetry; Anterior segment optical coherence tomography; AS-OCT; Specular microscopy; Glaucoma; Corneal pachymetry

INTRODUCTION

Corneal central thickness (CCT) is one of the most significant parameters in ophthalmology due to the role played by this parameter in the diagnosis, management, and prognosis of various anterior segment diseases including glaucoma, corneal ectasia diseases, refractive surgery, and postoperative assessments. Proper assessment of the corneal central thickness is crucial for the determination of intraocular pressure (IOP), since there will be an overestimation or underestimation of the IOP values measured by the Goldmann applanation tonometer. Increased CCT leads to a false elevation of the IOP values, while the thinner cornea might lead to underestimation of the IOP value, and thus affecting glaucoma diagnosis and management. Besides, studies have demonstrated that thin corneal central thickness is one of the independent risk factors for the development of primary open angle glaucoma (Brandt et al., 2001; Gordon et al., 2002; Thomas et al., 2000; Shields, 2011). Thus, proper measurement of the central corneal thickness should be an integral part of ophthalmic evaluation.

The ultrasound pachymetry (USP) has been long considered as the gold standard for clinical measurement of the CCT due to the high precision, reproducibility and accessibility of the test. However, the contact nature of the method requires the use of topical anesthetic and direct application of the probe onto the cornea making the measurements subject to operator-induced variations, misaligning of the probe, corneal indentation and even possible risks of the injury of the corneal epithelium and transfer of infection (Miglior et al., 2004; Sadoughi et al., 2015; Tai et al., 2013). The technological advancement brought new non-invasive methods of CCT

measurement – anterior segment optical coherence tomography (AS-OCT) and specular microscopy. These techniques offer high-speed and high-resolution imaging of the cornea that does not involve contact of the probe with the eye surface. AS-OCT is based on low-coherence interferometry and allows to obtain highly precise cross-sectional images of the anterior segment of the eye including pachymetry data along with corneal and anterior chamber morphology (Kim et al., 2008; Ang et al., 2018; Ayala & Strandås, 2015). Specular microscopy initially designed for endothelial cell evaluation also allows to get the accurate measurements of the CCT while providing the additional morphological information about the cornea (Chaurasia & Vanathi, 2021; Krashmer, 2011).
While some of the studies conducted to compare different pachymetric devices, there are substantial variations in the measurement of the corneal thickness due to the variation in imaging technology, calibration, acquisition algorithm, and patient-related factors. Various researchers have demonstrated high correlations between ultrasonic pachymetry, AS-OCT and Specular microscopy, but at the same time, they also identified that there were significant systematic differences in absolute CCT values. Hence, this indicated that these different devices were not entirely interchangeable in clinical practice (Khaja et al., 2015; Babbar et al., 2017; González-Pérez et al., 2018; Gokcinar et al., 2019). Similar findings have also been presented by Beutelspacher et al. (2011), Binnawi et al. (2019), Doğan and Ertan (2019), and Rajput et al. (2015), where the level of agreement was demonstrated among optical and ultrasonic pachymetry devices. As the measurement of the CCT has an impact on the detection of glaucoma, refractive surgery, and follow-up, it is important to evaluate the agreement among different modalities before using them interchangeably. Thus, the current study will help in determining the agreement among three of the most commonly used modalities: Ultrasound Pachymetry, Anterior Segment Optical Coherence Tomography (AS-OCT) and Specular Microscopy for CCT measurement.
The current research aims to compare the measurements between Ultrasound Pachymetry, Anterior Segment Optical Coherence Tomography (AS-OCT) and Specular Microscopy in their usage for central corneal thickness measurement. The purpose is to investigate whether the measurements are consistent in the measurements made using these methods and whether the methods can be considered interchangeable. These results will be helpful for physicians in choosing pachymetric instruments in glaucomatous and refractive surgeries as well as in general corneal examination. This research contributes to the understanding of reliability, reproducibility and clinical value of pachymetry.

METHODOLOGY

Study area: Study was undertaken in CIMS Chikkamagaluru.
Source of data: Patients above 40 years of age with clinically normal cornea who had attended general ophthalmology OPD.
Study duration: The study was conducted from October 2022 to March 2023.
Study design: Hospital based observational cross - sectional study
Sample size: 355
Using reference mean (d) and standard deviation (σ) from study carried out by Nesrin Buyuktortop and et al we derived the sample size as follows
Sampling technique: Convenience sampling.
Inclusion criteria -
Patients with best corrected visual acuity 20/20 or better.
Absence of ocular pathology by history and dilated eye examination.
CCT corrected IOP of less than 21mmHg.
Exclusion criteria -
History of ocular surgeries (including refractive surgeries).
History of trauma with primary repair.
Corneal ectasias and dystrophies.
Patients with glaucoma.
Patients on topical medications for any eye conditions.
Patients with only one eye.
Contact lens users.
Patients with high spherical (±3D) and cylindrical refractive errors (±4D).
Diabetic patients.
Patients who did not provide informed consent.

METHODOLOGY

This study has been performed following Institutional Scientific Committee approval and Institutional Ethics Committee approvals as per the guidelines for conducting experiments involving human subjects. The patients above 40 years of age who have clinically normal cornea visiting the General Ophthalmology OPD and satisfying the selection criteria were enrolled into the study after taking their informed written consent. After recording an elaborate ophthalmic history, a comprehensive ocular examination was done which comprised of visual acuity testing using Snellen's chart, anterior segment evaluation by slit lamp biomicroscopy and dilated fundus examination by using 78D/90D lenses. The central corneal thickness (CCT) was measured in three different ways

where non-contact techniques were used first followed by the contact technique (Ultrasound pachymetry) to avoid the effect of corneal contact in further measurements. Subsequently, the IOP was measured using Goldmann applanation tonometer. All the measurements were performed by the same experienced investigator within the same time period between 10:00 AM and 4:00 PM so as to maintain uniformity in measurements and minimizing the inter observer variability and diurnal variations. During the experiment all the necessary precautions as recommended for preventing COVID-19 infections were strictly followed.

Measurement Technique

The measurement of central corneal thickness was done using three different measurement techniques: Anterior Segment Optical Coherence Tomography (AS-OCT), Specular Microscopy, and Ultrasound Pachymetry. AS-OCT measurements were done using the DRI Triton Swept Source OCT with corneal adaptor module. Participants sat comfortably and kept their chin on the chin rest, while fixing on the internal target and positioning the corneal apex parallel to the scanning beam. Pachymetry scan protocol was used and the average central corneal thickness shown on the machine was taken as the reading. In specular microscopy, the measurement was performed using the Topcon SP-3000P Specular Microscope. Fixation on the target by the participants led to taking corneal endothelial images. Automated software was used to analyze the images and the CCT values were calculated. Ultrasound pachymetry was done using the Tomey Pachymeter 3000 after putting the proparacaine 0.5% eye drops for the topical anesthesia. Perpendicular placement of the handheld probe on the central cornea through mid-pupillary axis gave the mean CCT values of the ten consecutive readings done by the instrument. Three sequential readings of each participant for each eye were done for each technique after an interval of 15 seconds to allow for normal blinking. The reading with the best image quality in AS-OCT, the clearest endothelial image in specular microscopy and the reading with least standard deviation in ultrasound pachymetry were chosen for analysis.

Editorial verification before submission: The present analysis uses one-way ANOVA and Tukey’s post hoc test to compare mean CCT values. Because the manuscript is framed around agreement and interchangeability, the authors should verify the intended agreement analysis (for example, Bland–Altman analysis) and the handling of paired right- and left-eye measurements before final submission.

Statistical Analysis

The acquired data were fed into Microsoft Excel and then analyzed with the help of Statistical Package for the Social Sciences (SPSS) software, version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive analysis was carried out on the study variables and presented in terms of mean and standard deviation. The agreement and comparison of the central corneal thickness measurements taken by means of three different methods was done with the help of one-way Analysis of Variance (ANOVA). The pairwise comparison for any statistically significant differences between individual methods was done with the help of Tukey’s test. Fisher's test was done where applicable to compare the categorical variables.

RESULTS

Table 1: Age distribution

Age group (years)	Number of subjects	Percentage (%)
40 - 45	156	43.9
45 - 50	85	23.9
50 - 55	52	14.6
55 - 60	33	9.3
60 - 65	24	6.8
65 - 70	5	1.5

Mean age = 48.64 ± 7.01 years

Most of the people were in the age group of 40 - 45 years (nearly 43.9 %) whereas 23.9 % were in the age group of 45 - 50 years. Only 17 % were in the age group of above 55 years.

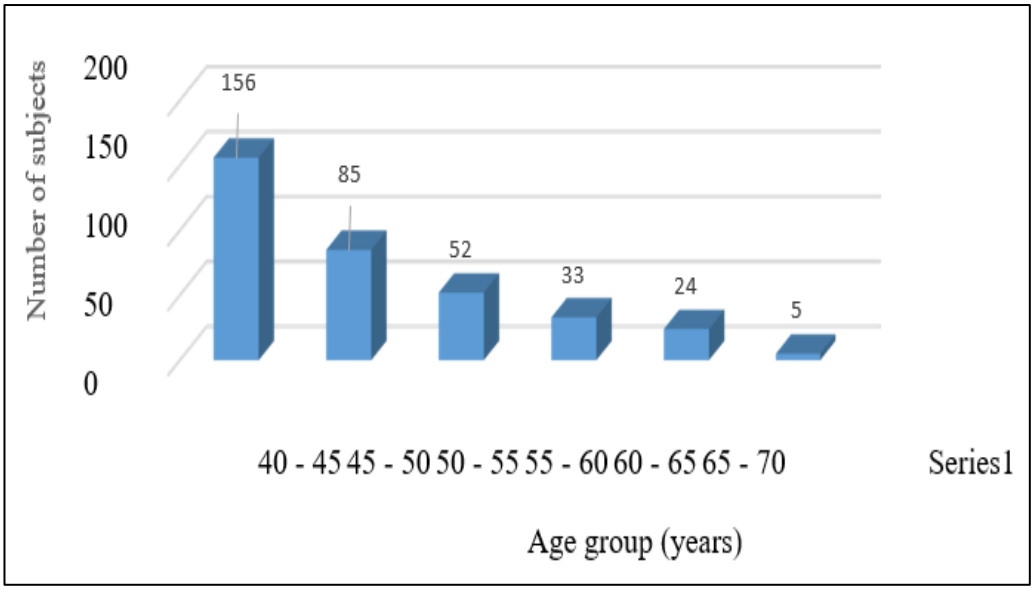

Fig.1. Age distribution

Table 2: Gender Distribution

Gender	Number of subjects	Percentage (%)
Male	154	43.4
Female	201	56.6
Total	355	100.0

Females were more in number as compared to males in our study. Among 355 subjects, 154 (43.4 %) were males and 201 (56.6 %) were females.

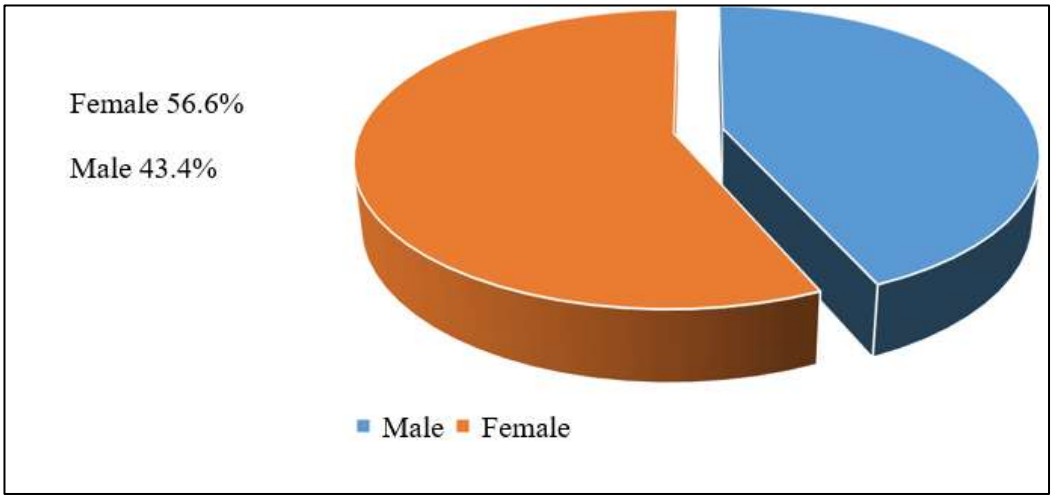

Fig. 2. Gender distribution

Table 3: Comparison of mean CCTs among three methods - Right eye (RE)

Method	Mean value (µm)	Standard deviation (SD)	Minimum (µm)	Maximum (µm)
AS OCT	512.21	25.26	407.0	579.0
Specular microscopy	504.73	25.36	401.0	573.0
Ultrasound pachymetry	523.34	25.51	425.0	601.0

F value	48.29	P < 0.001	

In RE, mean CCT was 523.34 µm with ultrasound pachymetry, 512.21 µm with AS OCT and 504.73 µm with specular microscopy.
While comparing the mean CCT among three methods, we could see that, the mean value was maximum for ultrasound pachymetry followed by AS OCT and minimum for specular microscopy. The difference among the three methods was statistically significant (one-way ANOVA, $p < 0.001$).
The box plot illustrates the distribution of CCT measurements obtained using the three methods.

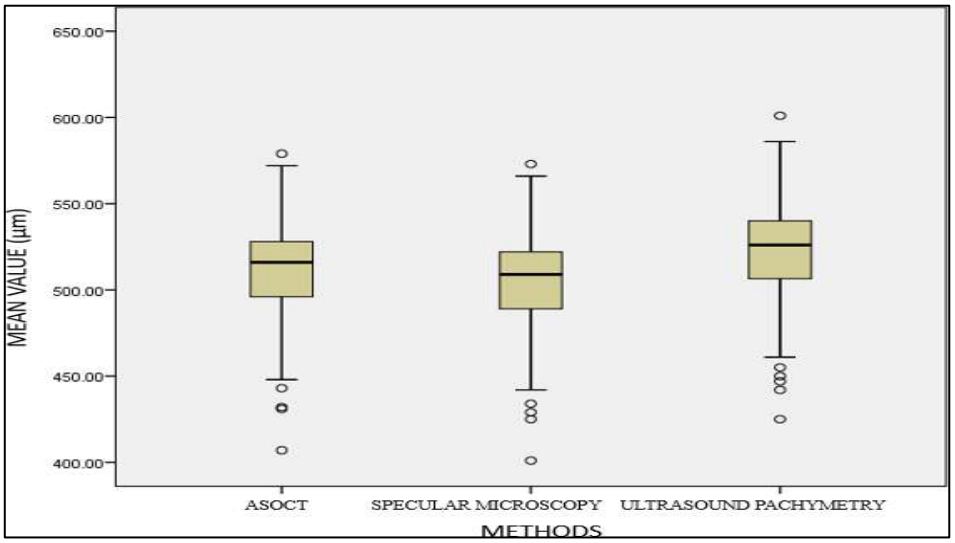

Fig. 3. Comparison of mean CCT among three methods – RE

Table 4: Intercomparison among methods – RE

Intercomparison of	with	Mean difference (µm)	P value
AS OCT	Specular microscopy	7.47	< 0.001
	Ultrasound pachymetry	11.12	< 0.001
Specular microscopy	Ultrasound pachymetry	18.6	< 0.001

By applying Tukey's test, we could see the intercomparison among these methods. In case of RE, the mean difference of CCT between specular microscopy and ultrasound pachymetry was more (18.6 µm) and found to be statistically significant ($p < 0.001$).
Similarly, the difference of CCT between AS OCT and ultrasound pachymetry was found to be 11.12 µm and the difference was minimum between AS OCT and specular microscopy (7.47 µm) but in both cases, difference was found to be statistically significant ($p < 0.001$).

Table 5: Comparison of mean CCT among three methods - Left eye (LE)

Methods	Mean value (µm)	SD	Minimum (µm)	Maximum (µm)
AS OCT	512.88	24.9	420.0	580.0
Specular microscopy	505.96	24.93	416.0	576.0
Ultrasound pachymetry	525.97	25.49	437.0	601.0
F value	58.148	P < 0.001		

In LE, mean CCT was 525.978 µm with ultrasound pachymetry, 512.882 µm with AS OCT and 505.963 µm with specular microscopy.
While comparing the mean CCT of LE among three methods, we could see that, the mean CCT value was maximum with ultrasound pachymetry followed by anterior segment OCT and minimum with specular microscopy. The difference among the three methods was statistically significant (one-way ANOVA, $p < 0.001$).

The box plot illustrates the distribution of CCT measurements obtained using the three methods.

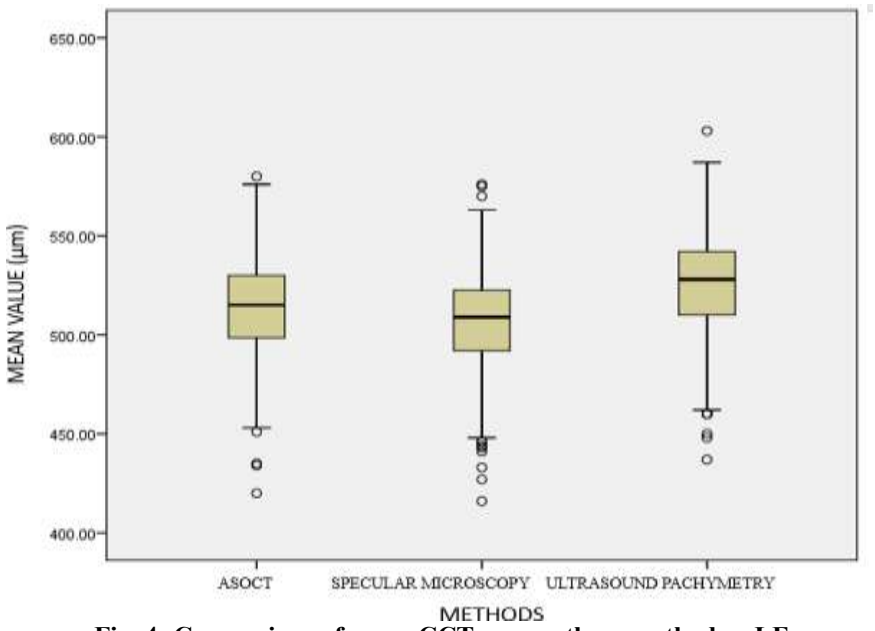

Fig. 4: Comparison of mean CCT among three methods – LE

Table 6: Intercomparison among methods – LE

Intercomparison of	With	Mean difference (µm)	P value
AS OCT	Specular microscopy	6.91	< 0.001
	Ultrasound pachymetry	13.09	< 0.001
Specular Microscopy	Ultrasound pachymetry	20.01	< 0.001

By applying Tukey's test, we could see the intercomparison among these methods. In case of LE also, the mean difference of central corneal thickness between specular microscopy and ultrasound pachymetry was more (20.01 µm) and found to be statistically significant ($p < 0.001$). Similarly, the difference of CCT between AS OCT and ultrasound pachymetry was found to be 13.09 µm and the difference was minimum between AS OCT and specular microscopy (6.91 µm) but in both the cases, difference was found to be statistically significant ($p < 0.001$).

Table 7: Comparison of mean CCT among three methods – both eyes (BE)

Methods	Mean value (µm)	SD	Minimum (µm)	Maximum (µm)
AS OCT	512.54	25.08	407	580
Specular microscopy	505.35	25.14	401	576
Ultrasound pachymetry	524.66	25.50	425	603
F value	53.18	P < 0.001		

In both eyes, mean CCT was 524.66 µm with ultrasound pachymetry, 512.54 µm with AS OCT and 505.35 µm with specular microscopy.
While comparing the mean CCT of BE among three methods, we could see that the mean value was maximum with ultrasound pachymetry followed by AS OCT and minimum with specular microscopy. The difference among the three methods was statistically significant (one-way ANOVA, $p < 0.001$).

The box plot illustrates the distribution of CCT measurements obtained using the three methods.

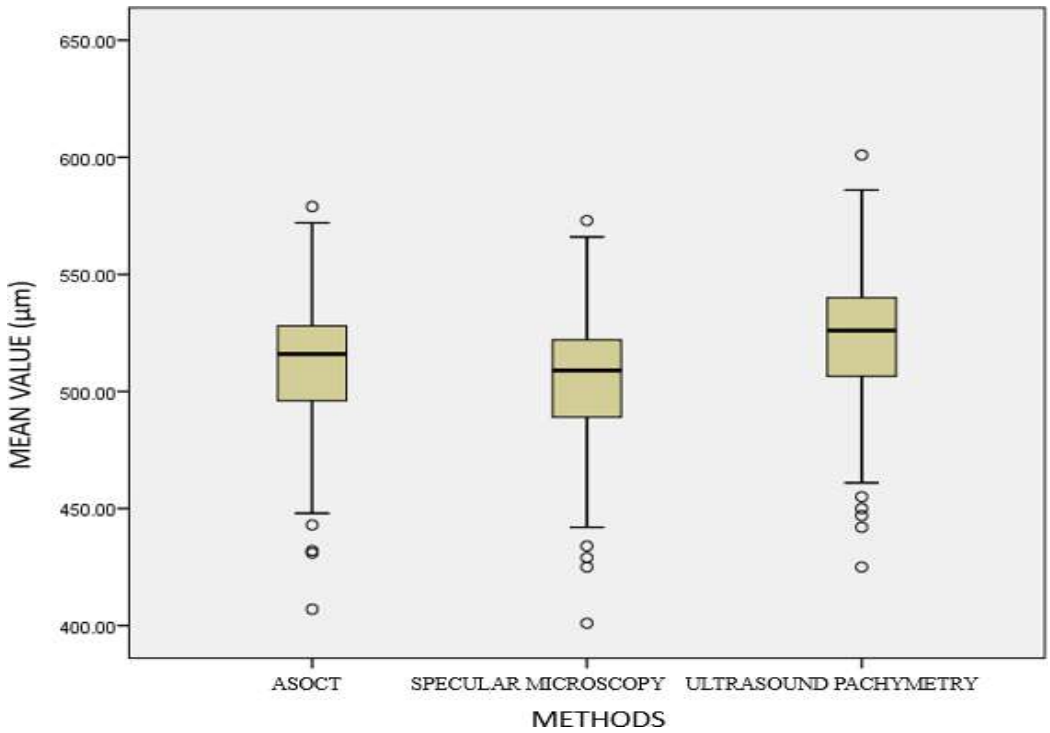

Fig. 5 Comparison of CCT among three methods – BE

Table 8: Intercomparison among methods - BE

Intercomparison of	with	Mean difference (µm)	P value
AS OCT	Specular microscopy	7.19	< 0.001
	Ultrasound pachymetry	12.11	< 0.001
Specular Microscopy	Ultrasound pachymetry	19.31	< 0.001

By applying Tukey's test, we could see the intercomparison among these methods. In case of both eyes also, the mean difference of CCT between specular microscopy and ultrasound pachymetry was more (19.31 µm) and found to be statistically significant ($p < 0.001$). Similarly, the difference of CCT between AS OCT and ultrasound pachymetry was found to be 12.11 µm and the difference was minimum between AS OCT and specular microscopy (7.19 µm) but in both cases, difference was found to be statistically significant ($p < 0.001$).

DISCUSSION

Agreement between three different pachymetric techniques for measuring central corneal thickness was assessed in the current research among 355 subjects with normal corneas, aged above 40 years. The age mean for the subjects in the study was 48.64 ± 7.01 years, with 43.9% of the study population falling within the 40–45-year age category and a slightly higher prevalence of females (56.6%). Previous studies conducted on pachymetric devices among normal eyes have included patients with demographic features similar to those of the current study (Tai et al., 2013; Babbar et al., 2017; Gokcinar et al., 2019). The mean central corneal thickness measurements recorded in the current research indicated a higher mean value for ultrasound pachymetry (524.66 ± 25.50 µm), followed by AS-OCT (512.54 ± 25.08 µm), and the lowest values were obtained with specular microscopy (505.35 ± 25.14 µm). The results are consistent with the studies carried out by Khaja et al. (2015), González-Pérez et al. (2018), Doğan and Ertan (2019), and Binnawi et al. (2019), which reported that ultrasound pachymetry measures corneal thickness readings higher than optical imaging. Ultrasound pachymetry has been considered the gold standard for its direct contact with the cornea although a slight overestimation might be due to probe positioning, corneal compression or different acoustic velocity assumptions used by the device (Miglior et al., 2004; Mohan et al., 2007). On the other hand, optical technologies use light imaging technology and automated edge detection algorithms.

Further on, in the present research, statistically highly significant differences were shown for all three methods for both the right and left eye (one-way ANOVA, $p < 0.001$). Pairwise comparisons made using Tukey's test show that the highest mean difference is between ultrasound pachymetry and specular microscopy (19.31 µm for both eyes), followed by ultrasound pachymetry and AS-OCT (12.11 µm), while the lowest difference can be observed

between AS-OCT and specular microscopy (7.19 μm). Similar results were reported by Kim et al. (2008) when comparing AS-OCT with ultrasound pachymetry. According to the researchers, the former method has good repeatability but provides underestimation of the CCT in comparison with ultrasound pachymetry. In a similar way, Ramesh et al. (2017) and González-Pérez et al. (2018) reported statistically significant differences between ultrasound pachymetry and AS-OCT although high correlations were observed. Babbar et al. (2017), Rajput et al. (2015), and Khaja et al. (2015) also compared these three methods of measuring the CCT and found that although they had highly significant correlations, the absolute CCT values were significantly different, suggesting that these devices should not be interchangeably used without taking into account the systematic bias. In a similar way, Gokcinar et al. (2019) and Beutelspacher et al. (2011) also observed significant inter-device variation although highly significant repeatability was provided, which can be explained by differences in measurement principles, image acquisition algorithm, and calibration technique.

In light of clinical practice, the results of the current study carry important implications for glaucoma treatment, surgical treatment planning, and longitudinal corneal measurements. Inasmuch as central corneal thickness is one of the critical predictors for the development of primary open-angle glaucoma and plays an integral role in the Goldmann applanation tonometry measurements, the reliability and reproducibility of CCT measurements are extremely important (Brandt et al., 2001; Gordon et al., 2002; Thomas et al., 2000; Sng et al., 2017). Despite the fact that the ultrasound pachymetry is still regarded as the gold standard due to its established reliability, the non-invasive methods of AS-OCT and specular microscopy provide many advantages, which include higher patient tolerance, lack of need for the use of topical anesthesia, lower risk of infection, quick image acquisition, and combined anterior segment structures or corneal endothelial layer assessment (Ang et al., 2018; Chaurasia & Vanathi, 2021). However, the statistically significant differences found in the current research mean that these measurement techniques cannot be considered interchangeable for clinical use. The same conclusions have been made by other researchers: Tai et al. (2013), Almubrad et al. (2011), Jr et al. (2010), Gonza et al. (2011), and Doğan and Ertan (2019) recommend that the patients undergoing the repeated examinations should be measured with the help of the same pachymeter in order to prevent possible measurement errors. Thus, the current study confirms the results of the previous research, according to which AS-OCT and specular microscopy can be regarded as reliable non-invasive techniques, whereas the ultrasound pachymetry should remain the clinical gold standard.

CONCLUSION

In the present study, the measurements of central corneal thickness (CCT) by Ultrasound Pachymetry (USP), Anterior Segment Optical Coherence Tomography (AS-OCT) and Specular Microscopy were evaluated in adults with normal corneas. The highest mean values of CCT were found in the case of ultrasound pachymetry, then came AS-OCT, and finally the lowest values were provided by specular microscopy. Significant differences were found in the right eye, left eye and in the both eyes together ($p < 0.001$). In addition, pairwise comparison revealed significant differences between each of the techniques, with the largest differences between ultrasound pachymetry and specular microscopy and the smallest ones between AS-OCT and specular microscopy. Thus, one can conclude that even though all these methods can provide reliable and repeatable measurements of central corneal thickness, systematic differences between them occur due to different measurement principles and technologies.

For clinical purposes, precise measurement of the CCT is crucial for glaucoma assessment, intraocular pressure interpretation, planning of refractive surgeries and evaluation of corneal diseases. Non-invasive techniques, including AS-OCT and specular microscopy, have such advantages as patient comfort, rapid image acquisition and low risk of infection, but these techniques cannot be regarded as equivalents to ultrasound pachymetry due to the differences in the measured values. Thus, when serial measurements of central corneal thickness are needed, the same device should always be used. In general, ultrasound pachymetry remains a gold standard, whereas AS-OCT and specular microscopy provide alternative non-invasive techniques.

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