Original Article

Repeatability of Corneal Densitometry Measurements using a Scheimpflug Camera in Healthy Normal Corneas

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Abstract

Purpose: To determine the repeatability of corneal densitometry measured by the Scheimpflug imaging system.

Methods: This cross-sectional study was conducted on photorefractive keratectomy candidates. One eye of each participant underwent imaging using Pentacam HR three times, 10 min apart. The repeatability of densitometry measurements was evaluated in four concentric annuli around the corneal apex and in different corneal depths. The repeatability of the measurements was evaluated using the intraclass correlation coefficient (ICC), repeatability coefficient (RC), and coefficient of variation (CV). The difference of repeatability between layers and zones was tested by tolerance index (TI).

Results: Sixty eyes of sixty patients with a mean age of 27.76 ± 3.93 years were studied. Half of the participants were female (n = 30, 50%). ICC was above 0.9 in all corneal parts. The posterior layer and central zones showed the least variability of densitometry measurements considering the CV values. The RC was 2.06, 1.17, and 0.92 in anterior, central, and posterior layers, respectively. The RC was 0.88, 0.71, 1.51, and 4.56 in 0–2, 2–6, 6–10, and 10–12 mm circles, respectively. Only the reliability of densitometry in 10–12 mm annulus was statistically lower than the central zone (TI = 0.71).

Conclusions: Corneal densitometry measurements provided by the Pentacam had good repeatability. The repeatability of densitometry measurements decreased from the center to the periphery (with an exception for 0–2 mm and 2–6 mm) and from the posterior to the anterior of the cornea. The reliability of the 10–12 mm zone was markedly less than other zones.

Keywords: Corneal densitometry, Reliability, Scheimpflug imaging system

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Introduction

Corneal transparency is important for the optical function of the cornea and is considered an index of corneal health.[1] Evaluation of the corneal transparency has several applications and indications, such as diagnosis of different corneal diseases and disorders like Fuchs endothelial dystrophy[2,3] and the healing process, and follow-up of patients undergoing refractive surgery like laser-assisted in-situ keratomileusis and photorefractive keratectomy (PRK).[4] It is also used to investigate the corneal effects of systemic diseases and drugs.[5,6] Different methods such as slit-lamp biomicroscopy, confocal microscopy, and Scheimpflug imaging are used to evaluate corneal transparency.[7-9] The Pentacam uses Scheimpflug principles to evaluate the transparency of the cornea and crystalline lens using the densitometry factor. This device presents the corneal densitometry in different corneal diameters in the anterior, central, and posterior cornea as a numerical index.[10]

Several studies have examined the repeatability of the Pentacam measurements of anterior segment parameters.

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The repeatability of some parameters such as keratometry, pachymetry, and anterior chamber data in normal and keratoconus patients has been investigated in previous studies. The majority of these studies found a high intrasubjective and intersubjective repeatability of anterior segment parameters in healthy subjects.[11-17] In addition, some studies evaluated the repeatability of the lens densitometry measurements of the Pentacam and reported high repeatability in normal and cataract eyes.[18]

Only two studies investigated the repeatability of corneal densitometry; however, they focused on patients with keratoconus and included a limited number of normal eyes as a control group.[19,20] The results of both studies showed that the repeatability of corneal densitometry was lower in keratoconus patients compared to nonkeratoconic eyes. In the Pahuja *et al*. study, repeatability was also lower in keratoconus patients that underwent collagen cross-linking (CXL) compared to keratoconus patients with a negative history of surgery.[19] This study recommended that the results of densitometry should be used with caution in post-CXL keratoconus patients. The other study was conducted by Kreps *et al*. which evaluated the repeatability of an extensive number of relevant indices with the Pentacam HR in various grades of keratoconus. They briefly deal with the repeatability of corneal densitometry and did not include the entire corneal tissue in their analysis.[20] There are no other studies on corneal densitometry repeatability in healthy eyes.

Since decreased corneal transparency is a diagnostic indicator for different corneal disorders and affects visual quality, it is very important to study the reliability of its measurements. This study was conducted to investigate the repeatability of the Pentacam measurements of corneal densitometry in normal eyes.

Methods

This cross-sectional study, which was part of a clinical trial,[21] was conducted on PRK candidates in the baseline phase before random allocation. The protocol of the study was approved by the Ethics Committee of Tehran University of Medical Sciences. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Tehran University of Medical Sciences Research Committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients signed a written informed consent (Ethics code: IR.TUMS.FARABIH.REC.1397.010).

Patients aged 21–40 years with a spherical equivalent of plano to −6.00 diopter (D), astigmatism ≤−2.00 D, and corrected distance visual acuity (CDVA) ≥20/20 were included in the study if their refractive error was stable (<0.5 D change) in the past year. Patients with any systemic diseases such as diabetes or thyroid disease, glaucoma and/or intraocular pressure ≥21 mmHg, keratoconus, corneal opacity, and dry eye; patients with a history of ocular surgery; and pregnant and lactating patients were not included in the study. Soft contact lens wearers were instructed to remove their lenses 1 week before imaging. The subjects who met the inclusion criteria underwent complete ophthalmic examinations including the measurement of uncorrected distance visual acuity, CDVA, subjective refraction, and slit-lamp biomicroscopy.

Pentacam imaging was done using the Pentacam HR (Oculus, Germany, version 1.22r. 9) in a windowless room with a light intensity of 3 lux. Repeatability was assessed in sixty eyes of sixty patients that were selected randomly. Imaging was done three times, 10 min apart, in each patient. In each stage, the patient was asked to fixate on a bright point in the middle of a blue line, blink several times, and then stop blinking until told otherwise. After completion of the imaging process, the joystick was retracted and the patient was given 10 min to rest. Then the device was set again and all the stages were repeated. Imaging was done by an experienced optometrist. Only images with a quality specification of "OK" were acceptable for analysis.

The "Cornea Densito" map shows the density of the corneal tissue in grayscale unit from 0 to 100, where 0 means maximum transparency and 100 means maximum scattering. The Cornea Densito map presents corneal densitometry in four annuli separately (central 0–2 mm, 2–6 mm, 6–10 mm, and 10–12 mm). Moreover, this map shows densitometry values according to depth in the anterior (120 µm), posterior (60 µm), and central layers (between anterior and posterior layers). Slit-lamp examination and fundoscopy were done in dilated condition after Pentacam imaging by an ophthalmologist.

Statistical analysis

The collected data were analyzed using the SPSS software version 23 (SPSS Inc., Chicago, Illinois, USA). The repeatability of corneal densitometry was investigated in different zones including the four annuli (0–2 mm, 2–6 mm, 6–10 mm, and 10–12 mm) in the anterior, posterior, and central layers as well as the entire cornea.

First, the mean and standard deviation of three readings were determined in each group. The repeated measures analysis of variance was done to compare the mean values. Considering the number of comparisons, the level of significance was reduced to 0.005 after Bonferroni correction.

The intrasession test–retest variability was also calculated. Then the within-subject standard deviation (S_w) was determined and multiplied by 2.77 to calculate the repeatability coefficient (RC). The RC is an indicator of repeated measurement error with lower values indicating better repeatability. The coefficient of variation (CV) was calculated by dividing the standard deviation by the mean value of the measurements and expressed as percentage. A lower value is more desirable with this index as well. The intraclass correlation coefficient (ICC) was calculated to assess the variance among repeated measurements. The ICC value can

be between 0 and 1 which 1 indicates no difference in the variance of repeated data and shows a strong correlation.[14] The tolerance index (TI) was calculated for examining the statistically significant difference of the repeatability measurements between zones and layers. TI >0.24 (cut-off values proper to the study sample size) means that there is a statistically significant difference.[22]

Results

Sixty eyes of sixty patients, of whom 30 individuals (50%) were female, were studied. The mean age of the participants was 27.76 ± 3.93 (range, 22–36 years). Table 1 shows the characteristics of participants. The mean densitometry of three measurements is demonstrated in Table 2. Table 3 shows the repeatability of corneal densitometry values in different corneal depths and concentric annuli around the apex. The ICC was above 0.9 in all corneal parts. According to the CV index, the variability of densitometry measurements increased from the center to the periphery of the cornea, and the posterior layer showed the least variability compared to the anterior and central layers.

The highest and lowest repeatability of the corneal densitometry values (based on RC index) were observed in the posterior and anterior layers, respectively. However, the difference in repeatability of central and posterior layers compared with the anterior layer was not statistically significant (TI = −0.24 and TI = −0.35), respectively. According to the concentric annuli around the apex, the 2–6 mm circle had the highest repeatability (the lowest RC), followed by the 0–2 mm, 6–10 mm, and 10–12 mm. Nevertheless, only the difference of repeatability in the zone 10–12 mm compared with the central zone (0–2 mm) was statistically significant (TI = 0.71).

Figure 1 demonstrates an example of corneal densitometry map of Pentacam.

Discussion

Considering the extensive use of the Pentacam in anterior segment evaluations, the measured parameters should have high repeatability to be reliable. The Pentacam calculates corneal densitometry quantitatively using the backscattered light in a fast and noncontact process and presents it as a Cornea Densito map. The densitometry data may serve as an objective index of corneal transparency in different conditions such as assessment and follow-up of corneal opacities.[23] Since the measurements of a device should have acceptable repeatability in order to be reliable, the repeatability of the corneal densitometry values measured by the Pentacam should be evaluated. Pahuja *et al*. investigated the corneal densitometry values in normal, keratoconus, and postcollagen CXL groups.[19] The results showed that the repeatability of corneal densitometry was lower in keratoconus patients compared to nonkeratoconic eyes. On the other hand, it was also lower in keratoconus patients that underwent collagen CXL compared to keratoconus patients with a negative history of surgery. This study recommended that the results of densitometry should be used with caution in post-CXL keratoconus patients since the measurements may not be reliable. Kreps *et al*. reported that densitometry repeatability in the central 0–2 mm annulus of the anterior layer was lower in keratoconus eyes compared to controls.[20] The present study was conducted on normal subjects. The ICC was above 0.9 in all corneal depths and diameters, indicating a high correlation between densitometry values. According to the concentric annuli around the apex, the 2–6 mm circle had the highest and the 10–12 mm annulus had the lowest repeatability. Ní Dhubhghaill *et al*. found similar results in eight eyes.[10] The highest mean value of densitometry was in the 10–12 mm annulus in this study, and the authors attributed this finding to the normal variation in corneal diameter. Since the corneal diameter is different in different people, the densitometry values measured in corneas with a diameter of <12 mm may include part of the limbus and sclera, and considering the higher light backscatter in these parts, the value of the densitometry will be higher too. This finding was recently confirmed in a study investigating densitometry values in PRK candidates using the Pentacam. In this study, the highest densitometry value was seen in the 10–12 mm annulus followed by the 0–2 mm, 6–10 mm, and 2–6 mm annuli.[24] Pahuja *et al*. did not include the data of the 10–12 mm annulus in the analysis, but the best annulus in terms of repeatability in normal subjects was the 0–2 mm circle.[19] Kreps *et al*. only evaluated the repeatability of 0–2 mm and 2–6 mm annuli and reported better repeatability in 2–6 mm radius.[20]

In the present study, according to the corneal depth, densitometry measurements had the highest repeatability in the posterior layer compared to the central and anterior layers, which was consistent with previous studies.[10,19] However, Pakbin *et al*.[24] and Asrar *et al*.[25] reported the lowest and highest mean densitometry values were in the posterior and anterior layers, respectively.

According to the studies, lower repeatability may be related to higher densitometry values. In other words, repeatability may be lower in corneal parts with higher densitometry values. The results of the study by Pahuja *et al*. also indicate the same conclusion since the repeatability index was lower

Table 1: Subject characteristics

	Mean±SD (range)
Age (years)	27.76±3.93 (22-36)
UDVA (logMAR)	0.75±0.38 (0.30-1.40)
CDVA (logMAR)	0±0 (0-0)
Sphere (D)	−2.84±1.20 (−5.50 - −0.75)
Cylinder (D)	−0.73±0.57 (−2.00-0)
Spherical equivalent (D)	−3.20±1.24 (−5.75 - −1.00)
Minimum K (D)	42.81±1.13 (40.70-44.80)
Maximum K (D)	43.75±1.19 (41.30-45.80)

CDVA: Corrected distance visual acuity, SD: Standard deviation, UDVA: Uncorrected distance visual acuity, D: Diopter, K: Keratometry

Table 2: The corneal densitometry values of three measurements and average

	Mean±SD (range)				
	Time 1 (n=60)	Time 2 (n=60)	Time 3 (n=60)	Mean difference	Average (n=60)
Anterior 120 µm (GSU)					
0-2 mm	23.68±1.65 (20.00-27.70)	23.52±1.47 (20.30-26.90)	23.49±1.41 (20.10-26.80)	0.13±0.50	23.56±1.50 (20.00-27.70)
2-6 mm	20.98±1.62 (16.90-24.80)	20.85±1.44 (18.00-24.70)	20.78±1.38 (18.10-24.20)	0.13±0.40	20.87±1.48 (16.90-24.80)
6-10 mm	18.64±4.26 (13.80-40.10)	18.48±4.09 (13.80-38.30)	18.31±3.73 (14.10-34.90)	0.22±0.70	18.47±4.01 (13.80-40.10)
10-12 mm	27.01±8.32 (9.50-49.80)	27.37±7.95 (13.80-46.40)	27.08±7.50 (14.10-46.80)	−0.05±3.30	27.15±7.88 (9.50-49.80)
Total	21.67±3.00 (17.00-33.50)	21.61±2.92 (17.10-32.90)	21.47±2.65 (17.50-29.10)	0.13±0.80	21.58±2.84 (17.00-33.50)
Center (GSU)					
0-2 mm	13.93±0.71 (12.40-15.60)	13.94±0.74 (12.50-15.60)	13.95±0.77 (12.50-16.40)	−0.01±0.30	13.94±0.74 (12.40-16.40)
2-6 mm	12.36±0.84 (10.90-16.20)	12.37±0.84 (11.30-16.20)	12.37±0.81 (11.30-16.20)	0±0.20	12.36±0.82 (10.90-16.20)
6-10 mm	11.93±2.94 (9.10-26.70)	11.90±2.86 (9.30-26.00)	11.86±2.60 (9.30-23.70)	0.05±0.40	11.89±2.78 (9.10-26.70)
10-12 mm	17.29±4.65 (7.70-31.60)	17.59±4.44 (9.70-30.00)	17.73±4.33 (10.80-30.80)	−0.29±1.90	17.53±4.45 (7.70-31.60)
Total	13.31±1.86 (11.10-21.50)	13.35±1.77 (11.10-20.60)	13.36±1.67 (11.20-19.30)	−0.03±0.50	13.33±1.75 (11.10-21.50)
Posterior 60 µm (GSU)					
0-2 mm	12.55±0.70 (11.10-14.80)	12.55±0.73 (10.90-14.80)	12.55±0.72 (11.00-14.40)	0±0.30	12.55±0.71 (10.90-14.80)
2-6 mm	11.20±0.70 (10.20-13.60)	11.19±0.71 (10.10-14.20)	11.19±0.74 (10.10-13.80)	0±0.20	11.19±0.71 (10.10-14.20)
6-10 mm	11.18±2.23 (8.50-20.80)	11.21±2.30 (8.60-22.60)	11.19±2.21 (9.00-20.90)	−0.11±0.50	11.19±2.20 (9.00-23.00)
10-12 mm	14.24±2.85 (8.50 -23.50)	14.57±2.87 (8.20-22.90)	14.66±3.01 (9.10-23.10)	−0.28±1.20	14.49±2.90 (8.20-23.50)
Total	11.93±1.32 (10.10-16.60)	11.99±1.34 (10.10-17.80)	11.99±1.37 (10.30-17.20)	−0.04±0.30	11.97±1.33 (10.10-17.80)
Total thickness (GSU)					
0-2 mm	16.72±0.92 (14.70-18.70)	16.67±0.88 (14.60-18.60)	16.67±0.86 (14.70-18.30)	0.03±0.30	16.68±0.88 (15.00-19.00)
2-6 mm	14.84±0.97 (12.70-18.00)	14.81±0.92 (13.30-18.40)	14.78±0.89 (13.20-18.00)	0±0.20	14.81±0.92 (12.70-18.40)
6-10 mm	13.93±3.11 (10.80-29.20)	13.87±3.06 (11.00-29.00)	13.79±2.81 (11.00-26.50)	−0.03±0.90	13.86±2.98 (10.80-29.20)
10-12 mm	19.52±4.89 (8.60-32.70)	19.84±4.69 (11.40-31.40)	19.82±4.49 (12.20-31.80)	−0.20±1.90	19.72±4.67 (8.60-32.70)
Total	15.63±2.00 (13.00-23.80)	15.65±1.97 (13.00-23.80)	15.61±1.83 (13.20-21.70)	0.01±0.50	15.63±1.92 (13.00-23.80)

GSU: Grayscale unit, SD: Standard deviation

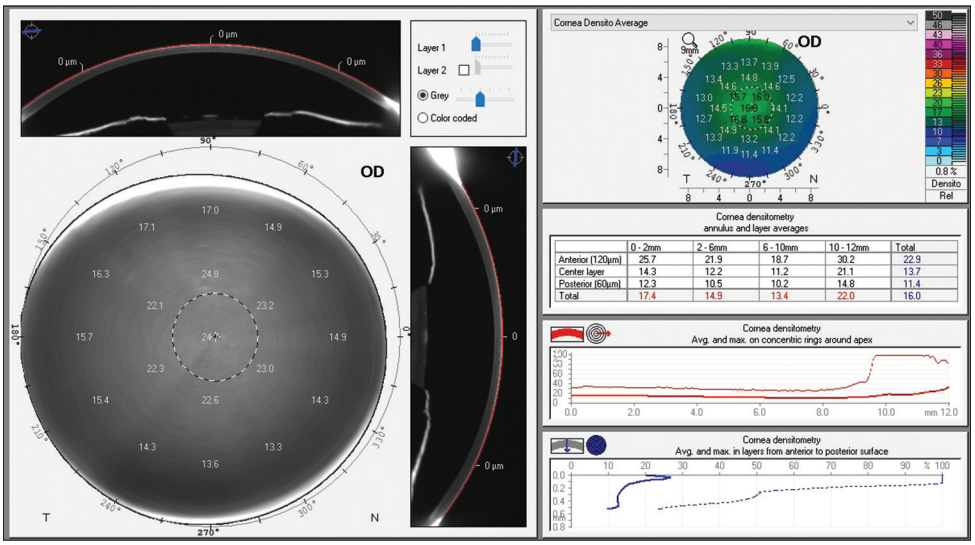

Figure 1: Corneal densitometry output of Pentacam

in keratoconus subjects, and it was even much worse in patients with a history of collagen CXL.[19] One of the common phenomena after CXL is corneal haze, which peaks 1 month after the operation, reaches a plateau within 3 months, and then reduces markedly until 12 months.[26] Shetty *et al*. conducted a study in patients that underwent CXL and found that the repeatability of corneal densitometry measurements was affected by post-CXL haze, especially anterior haze in the 0–2 mm region.[27]

The present study investigated the repeatability of corneal densitometry measurements in normal eyes. The lack of similar studies in normal subjects with this sample size is a strength of this study.

Table 3: Repeatability indices of corneal densitometry between three measurements

	ICC (95% CI)	RC	CV (%)
Anterior 120 µm (GSU)			
0-2 mm	0.969 (0.953-0.981)	1.25	6.24
2-6 mm	0.977 (0.964-0.985)	1.09	6.96
6-10 mm	0.984 (0.975-0.990)	2.45	21.48
10-12 mm	0.950 (0.924-0.969)	8.03	27.85
Total	0.976 (0.964-0.985)	2.06	12.96
Center (GSU)			
0-2 mm	0.947 (0.919-0.967)	0.77	5.08
2-6 mm	0.980 (0.969-0.987)	0.54	6.59
6-10 mm	0.990 (0.985-0.994)	1.30	23.33
10-12 mm	0.954 (0.930-0.971)	4.39	24.42
Total	0.980 (0.969-0.987)	1.17	12.99
Posterior 60 µm (GSU)			
0-2 mm	0.932 (0.895-0.957)	0.83	5.34
2-6 mm	0.965 (0.947-0.978)	0.61	6.21
6-10 mm	0.992 (0.988-0.995)	0.93	19.93
10-12 mm	0.954 (0.930-0.971)	2.89	19.24
Total	0.978 (0.967-0.986)	0.92	11.00
Total thickness (GSU)			
0-2 mm	0.953 (0.928-0.970)	0.88	5.11
2-6 mm	0.973 (0.958-0.983)	0.71	6.10
6-10 mm	0.989 (0.983-0.993)	1.51	21.39
10-12 mm	0.955 (0.931-0.972)	4.56	22.79
Total	0.980 (0.969-0.987)	1.28	12.14

CI: Confidence interval, CV: Coefficient of variation, GSU: Grayscale unit, ICC: Intraclass correlation coefficient, RC: Repeatability coefficient, SD: Standard deviation

This study was conducted on 20–40-year-old PRK candidates with moderate myopia, and patients with corneal diseases were not included; therefore, it does not represent the whole community and can be considered a study limitation.

In conclusion, corneal densitometry measurements in normal subjects have acceptable repeatability. The repeatability of corneal densitometry increases from the anterior layer to the posterior and from the periphery to the center. When using the data of corneal densitometry, it should be noted that the data of the 10–12 mm zone are less reliable than the data of other corneal regions; therefore, the data related to this annulus should be used with caution.

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Conflicts of interest

There are no conflicts of interest.

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