Corneal Densitometry: Repeatability in Eyes With Keratoconus and Postcollagen Cross-Linking

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Purpose: To assess the repeatability of densitometry, a measure of corneal haze, in the control (group I), keratoconic (group II), and postcollagen cross-linking (CXL, group III) eyes as measured on Scheimpflug imaging.

Methods: Densitometry values for 160 eyes of 160 patients (50 eyes of 50 patients in group I, 50 eyes of 50 patients in group II, and 60 eyes of 60 patients in group III) were obtained for the 0- to 2-mm, 2- to 6-mm, and 6- to 10-mm zones of the anterior (up to 120 μm), posterior (posterior 60 μm), and central (between the anterior and posterior) cornea. The repeatability of these values was assessed by within-subject standard deviation, coefficient of repeatability, and coefficient of variation.

Results: Range of within-subject standard deviation and coefficient of variation in the control group (0.2%–0.5% and 2%–4%, respectively) was significantly better (less variable) than those in the keratoconus group (0.4%–0.6% and 3%–5%). The same parameters in the post-CXL group (0.8%–3.8% and 7%–15%) were significantly worse (more variable) than that in the other 2 groups. The repeatability measures of densitometry were significantly worse in the central 0- to 2-mm zone compared with the other 2 zones and for the anterior region compared with the central and posterior regions of cornea in all the 3 groups.

Conclusions: Consequent to the low repeatability in post-CXL eyes, densitometry should be used with caution to gauge response to treatment and visual outcomes in treated keratoconus eyes.

Key Words: corneal densitometry, keratoconus, repeatability, collagen cross-linking

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Corneal imaging has evolved in the past few decades. Newer technology allows both qualitative and quantitative analysis of the entire cornea thereby allowing accurate monitoring of disease progression. Whereas anterior segment optical coherence tomography provides high-resolution cross-sectional images of the cornea, in vivo confocal microscopy provides cellular and subcellular images of the corneal tissue at various depths. Another technology, Scheimpflug photography, generates images of the anterior segment in 3 dimensions. It provides anterior and posterior surface topography of the cornea that is derived from true elevation measurements. It works with maximally possible depth of focus and minimal image distortion.[1]

The Oculus Pentacam (Oculus Inc, Wetzlar, Germany) uses the Scheimpflug principle to take up to 50 cross-sectional images of the entire anterior segment within 2 seconds.[2,3] It is a noncontact method and can assess the anterior corneal surface to the posterior lens surface in a single scan and simultaneously provide complete corneal pachymetry, corneal topography, and densitometry of cornea and lens (a measure of the scattering of the light), including opacities and anterior chamber analysis (depth, angle, and volume).[2,3] Corneal densitometry has been previously used to assess and quantify the degree of subepithelial corneal haze and stromal scarring in patients who have undergone refractive surgeries and keratoplasties.[4–6] It has also been used to assess corneal density in active and healed stages of bacterial keratitis,[7] postprimary pterygium excision,[8] corneal dystrophy,[9] keratoconus, and postcollagen cross-linking (CXL).[10–17]

Corneal haze after CXL is different from that after other procedures both in appearance (dust-like change) and natural history. It normally peaks between 1 and 3 months and diminishes over time, approaching but not reaching baseline at 1 year[11] Although the normative data of densitometry are established, the repeatability of corneal densitometry measured using the Pentacam in keratoconus or post-CXL has not been reported.[18] The aim of this study was to evaluate the repeatability of corneal density measurement of healthy subjects and in patients with keratoconus and those who have undergone CXL.

MATERIALS AND METHODS

This prospective study was approved by the ethics committee of Narayana Nethralaya Eye Hospital and was performed according to the tenets of the Declaration of Helsinki after obtaining a written consent from all patients. Patients between 18 and 35 years were enrolled for the study and divided into 3 groups:

Group I (Controls—50 eyes of 50 patients): This group included emmetropic patients with no history of contact lens use, previous trauma, ocular surgery, or comorbidities.

Group II (Keratoconus patients—50 eyes of 50 patients): This group included patients with keratoconus showing a superior inferior asymmetry of ≥1.5 diopters on topography along with clinical signs of ectasia. Those who had discontinued contact lens use (at least 3 wks prior for rigid gas permeable lenses) or were only spectacle users were included. Exclusion criteria included a history of prior ocular surgery or comorbidity and corneal scarring on slit-lamp examination.

Group III (Post-CXL patients—60 eyes of 60 patients): This group included patients with keratoconus without corneal scarring who had undergone accelerated CXL (epithelial-off, 365 nm, 9 mW/cm^2 for 10 min) at 6 months of follow-up. Those using contact lenses had been asked to discontinue the same at least 3 weeks before the examination. Patients with delayed epithelial healing and post-CXL keratitis were excluded from the study. The grades of keratoconus were comparable between group II and group III.

Patients in all 3 groups underwent a detailed ophthalmic examination including uncorrected distance visual acuity, corrected distance visual acuity, subjective refraction, and slit-lamp and dilated fundus examination. The Pentacam was used to acquire 3 good quality images for each eye by the same examiner in a dark room in a single session. The patient was asked to look at the fixation target when acquiring the scans to ensure centration. The images were analyzed using the add-on corneal densitometry software, which measures haze across a corneal diameter of 12 mm and expresses it in gray scale units ranging from 0 (optically clear cornea/no haze) to 100 (total corneal opacity). The 12-mm zone is further divided by concentric radial circles into 4 zones: 0 to 2 mm, 2 to 6 mm, 6 to 10 mm, and 10 to 12 mm, and based on the depth of the cornea into anterior (up to 120 µm), posterior (posterior 60 µm), and central (between the anterior and posterior zones). The repeatability of densitometry measurements in each of the zones except the 10- to 12-mm zone was analyzed. The peripheral 10 to 12 mm was excluded from the analysis, as this zone is already known to have very low repeatability.[18]

Statistical Analysis

Repeatability was assessed by within-subject standard deviation (Sw), coefficient of repeatability (CRw), and coefficient of variation (CVw). The Sw was calculated as the square root of the within-subject mean square error (the unbiased estimator of the component of variance because of random error) in a 1-way random effects model.[19] The CRw was calculated as 2.77 times Sw. The CVw was calculated as $100 \times$ Sw/overall mean. Standard error and confidence intervals for CVw were calculated based on the root mean square method as described by Bland and Altman.[20] Statistical analyses were performed using Stata version 12.1 (StataCorp, College Station, TX) statistical software.

RESULTS

One hundred sixty eyes of 160 patients (50 eyes of 50 patients in group I, 50 eyes of 50 patients in group II, and 60 eyes of 60 patients in group III) were included in the study. Mean age of the patients was 28.0 ± 4.5 years in group I, 26.3 ± 6.2 in group II, and 24.4 ± 3.7 in group III.

Table 1 shows the age and densitometry values in each of the groups. The densitometry values were comparable ($P > 0.05$ for all comparisons) between the control and the keratoconus groups, whereas the same in the post-CXL group were significantly higher than those in the other 2 groups ($P < 0.05$ for all comparisons).

Tables 2–4 show the repeatability measures (Sw, CRw, and CVw respectively) of densitometry in the 3 groups. Repeatability measures in the control group (group I) were significantly better (less variable) than those in the keratoconus group (group II) (nonoverlapping 95% confidence limits). Repeatability measures in the post-CXL group (group III) were significantly worse (more variable) than those in the control and keratoconus groups. The repeatability measures of densitometry were significantly worse in the central 0- to 2-mm zone compared with the 2- to 6-mm zone. Repeatability measures in the 2- to 6-mm zone were worse than those in the 6- to 10-mm zone. The repeatability measures of densitometry were significantly worse in the anterior region compared with those in the central and posterior region of the cornea in all the 3 groups. Figure 1 shows the representative scans in a control (Fig. 1A), keratoconic (Fig. 1B), and post-CXL subject (Fig. 1C).

DISCUSSION

Corneal collagen CXL has been successful in stabilizing the progression of keratoconus.[21] In the early postoperative period, there is often a reduction in visual acuity

TABLE 1. Age and Densitometry Measurements in the 3 Groups

	Control Group, 50 Eyes	Keratoconus Group, 50 Eyes	Post-CXL Group, 60 Eyes
Age, yrs	28.0 ± 4.5	26.3 ± 6.2	24.4 ± 3.7
Anterior, mm			
0–2	13.1 ± 1.2	14.6 ± 2.5	27.8 ± 7.6
2–6	12.2 ± 1.1	13.0 ± 1.3	22.3 ± 4.4
6–10	11.6 ± 1.3	11.7 ± 1.2	17.4 ± 2.6
Central, mm			
0–2	9.9 ± 0.9	10.5 ± 1.4	15.9 ± 3.2
2–6	9.2 ± 0.9	9.6 ± 0.9	13.2 ± 1.5
6–10	9.1 ± 1.0	9.2 ± 1.0	12.1 ± 2.6
Posterior, mm			
0–2	8.7 ± 0.9	8.8 ± 0.9	11.7 ± 1.9
2–6	8.3 ± 0.9	8.6 ± 0.8	11.2 ± 1.0
6–10	8.9 ± 1.1	8.9 ± 1.0	11.0 ± 2.8
Total, mm			
0–2	10.5 ± 1.0	11.3 ± 1.5	18.5 ± 3.8
2–6	9.9 ± 0.9	10.4 ± 0.9	15.5 ± 2.2
6–10	9.9 ± 1.1	9.9 ± 1.1	13.4 ± 2.0

TABLE 2. Within-Subject Standard Deviation of Densitometry Measurements (95% Confidence Intervals of the Estimates Are Shown in Parentheses)

|	Control Group	Keratoconus Group	Post-CXL Group
Anterior, mm			
0–2	0.5 (0.4–0.6)	0.6 (0.5–0.7)	3.8 (3.4–4.4)
2–6	0.4 (0.3–0.4)	0.5 (0.4–0.5)	2.3 (2.0–2.6)
6–10	0.3 (0.2–0.3)	0.5 (0.4–0.5)	1.6 (1.4–1.8)
Central, mm			
0–2	0.2 (0.2–0.2)	0.5 (0.4–0.6)	1.3 (1.1–1.4)
2–6	0.2 (0.2–0.2)	0.4 (0.3–0.4)	1.0 (0.8–1.1)
6–10	0.2 (0.2–0.2)	0.4 (0.4–0.5)	1.3 (1.1–1.5)
Posterior, mm			
0–2	0.2 (0.2–0.3)	0.5 (0.4–0.5)	1.0 (0.9–1.1)
2–6	0.2 (0.2–0.2)	0.4 (0.3–0.4)	0.8 (0.7–0.9)
6–10	0.2 (0.2–0.2)	0.5 (0.4–0.5)	0.9 (0.8–1.0)
Total, mm			
0–2	0.2 (0.2–0.3)	0.6 (0.5–0.6)	1.9 (1.7–2.1)
2–6	0.2 (0.1–0.2)	0.4 (0.4–0.5)	1.3 (1.1–1.4)
6–10	0.2 (0.1–0.2)	0.5 (0.4–0.5)	1.6 (1.4–1.8)

TABLE 4. Within-Subject Coefficient of Variation of Densitometry Measurements (95% Confidence Intervals of the Estimates Are Shown in Parentheses)

	Control Group	Keratoconus Group	Post-CXL Group
Anterior, mm			
0–2	0.04 (0.00–0.06)	0.04 (0.02–0.05)	0.15 (0.09–0.20)
2–6	0.03 (0.00–0.05)	0.03 (0.02–0.04)	0.11 (0.06–0.15)
6–10	0.02 (0.01–0.03)	0.04 (0.03–0.04)	0.10 (0.05–0.13)
Central, mm			
0–2	0.02 (0.01–0.02)	0.05 (0.02–0.06)	0.09 (0.05–0.11)
2–6	0.02 (0.01–0.02)	0.04 (0.03–0.05)	0.07 (0.04–0.10)
6–10	0.02 (0.01–0.02)	0.05 (0.03–0.06)	0.09 (0.00–0.13)
Posterior, mm			
0–2	0.03 (0.02–0.03)	0.05 (0.04–0.06)	0.09 (0.07–0.10)
2–6	0.03 (0.02–0.03)	0.05 (0.03–0.05)	0.07 (0.05–0.09)
6–10	0.02 (0.01–0.03)	0.05 (0.03–0.07)	0.08 (0.02–0.11)
Total, mm			
0–2	0.02 (0.01–0.03)	0.05 (0.03–0.06)	0.11 (0.07–0.14)
2–6	0.02 (0.01–0.02)	0.04 (0.03–0.05)	0.09 (0.05–0.11)
6–10	0.02 (0.01–0.02)	0.05 (0.03–0.06)	0.10 (0.05–0.14)

secondary to changes in the epithelium or corneal topography.[21–23] There is also a decrease in the corneal transparency secondary to alterations in the optical density of the stroma during the first months after CXL which in turn causes increased scattering of light.[11] Backward-scattered light can be observed as haze on the slit lamp or on Scheimpflug imaging. Whereas slit-lamp assessment of haze is subjective, densitometry on the Pentacam is an objective measurement.

Risk factors for the development of corneal haze after CXL include preoperative markedly visible Vogt striae and corneal scars; uncontrolled intraoperative stromal dehydration leading to a reduction in the intraoperative corneal thickness; thin corneas with a minimum corneal thickness under

TABLE 3. Within-Subject Coefficient of Repeatability of Densitometry Measurements (95% Confidence Intervals of the Estimates Are Shown in Parentheses)

	Control Group	Keratoconus Group	Post-CXL Group
Anterior, mm			
0–2	1.4 (1.2–1.6)	1.7 (1.5–2.0)	10.6 (9.4–12.1)
2–6	1.1 (0.9–1.2)	1.3 (1.1–1.5)	6.3 (5.5–7.1)
6–10	0.8 (0.7–0.9)	1.2 (1.1–1.4)	4.3 (3.8–4.9)
Central, mm			
0–2	0.5 (0.5–0.6)	1.3 (1.2–1.5)	3.5 (3.1–3.9)
2–6	0.5 (0.4–0.5)	1.0 (0.9–1.1)	2.6 (2.3–3.0)
6–10	0.5 (0.4–0.6)	1.1 (1.0–1.3)	3.6 (3.2–4.1)
Posterior, mm			
0–2	0.7 (0.6–0.8)	1.3 (1.1–1.5)	2.7 (2.4–3.1)
2–6	0.6 (0.5–0.7)	1.1 (0.9–1.2)	2.2 (1.9–2.5)
6–10	0.6 (0.5–0.6)	1.3 (1.1–1.5)	2.5 (2.2–2.9)
Total, mm			
0–2	0.6 (0.5–0.7)	1.5 (1.3–1.8)	5.2 (4.6–5.9)
2–6	0.5 (0.4–0.6)	1.2 (1.0–1.3)	3.5 (3.1–4.0)
6–10	0.5 (0.4–0.6)	1.3 (1.1–1.5)	4.5 (4.0–5.1)

400 mm at the time of treatment; patient age more than 35 years; the presence of activated keratocytes in the anterior stroma on preoperative in vivo confocal microscopy; forwarded defocus of UV-A source on the corneal plane; lack of administration of riboflavin 0.1% solution during UV-A irradiation, or excessive intraoperative riboflavin–dextran 20% solution administration (causing intraoperative stromal dehydration); patient noncompliance with postoperative therapy; postoperative infections or therapeutic contact lens intolerance; hypoxia and the presence of Langerhans cells seen on in vivo confocal microscopy after therapeutic contact lens removal.[23–26]

Because the effect of CXL is confined to the area of irradiation,[27] it is possible that haze is restricted to this area with differences in the stromal opacity evident at different depths and concentric zones of the stroma. Hence, we assessed the repeatability of densitometry in the 0- to 2-mm, 2- to 6-mm, and 6- to 10-mm zones of the anterior, mid, and posterior stroma of the cornea.

Repeatability or test–retest reliability is the variability in measurements taken by a single person or instrument, under the same conditions within a short period of time, over which the underlying value can be considered to be constant. Factors affecting the repeatability of measurements include poor instrument quality, noncalibration, inadequate methods of data collection, and operator inefficiency or inexperience. Although a high repeatability of any instrument's measurement is an indication of its precision, measurements with a low repeatability should be interpreted with caution. Management of various medical conditions, including those in ophthalmology, relies on measurements obtained from several instruments. Because readings may be taken over different machines and several times on each during the course of disease management, the reliability and comparability of these measurements are of paramount importance.
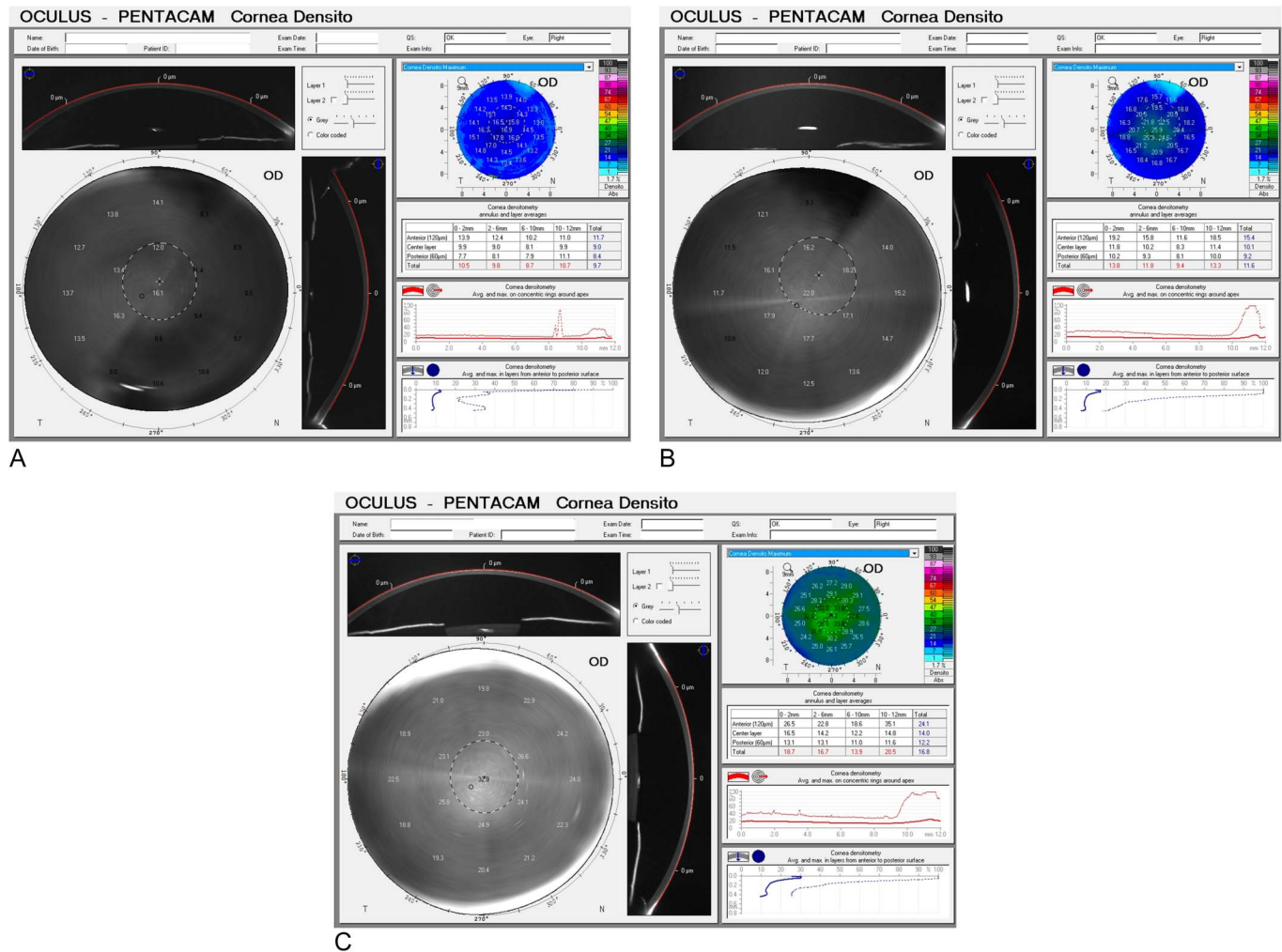
FIGURE 1. The average densitometry values in different annuli around the apex and in 3 different layers of the cornea in a representative control eye (A), keratoconus eye (B), and postcollagen CXL eye (C).

The challenge posed by any new diagnostic modality is to ensure comparability with data generated by other similar instruments and with data generated at different time points in clinical practice. Although this comparability is not always possible, a high repeatability ensures reliability of the data. Although multiple studies have evaluated the change in corneal densitometry values over time after CXL, the repeatability of densitometry measurements in keratoconus or post-CXL has not been previously reported in the literature. In our study, we found that the repeatability of densitometry measurements in post-CXL eyes was significantly worse than that in control and keratoconic eyes. The repeatability measures were worse in the central 0- to 2-mm zone and significantly worse in the anterior region compared with the central and the posterior regions of cornea. Although we assessed the repeatability of densitometry at 6 months, it will be interesting to study the same in the immediate postoperative period when more haze is expected and at long-term follow-up. The haze of the anterior cornea may affect posterior region data acquisition and its interpretation. This is a potential limitation and needs further study.

Considering the low repeatability we saw in post-CXL eyes, densitometry should be used with caution to gauge response to treatment and visual outcomes in treated keratoconus eyes. On the other hand, variable densitometry itself may have an influence on the other readings of the Pentacam and future studies need to assess this.

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