

Does Corneal Collagen Cross-Linking Effect the Repeatability and Reliability of Scheimpflug Imaging in Keratoconus?

Korneal Kollajen Çapraz Bağlama Keratokonus Hastalarında Scheimpflug Görüntülemenin Tekrarlanabilirlik ve Güvenilirliğini Etkiler mi?

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ABSTRACT Objective: The aim of this study was to assess the repeatability and reliability of measurements obtained with Pentacam in keratoconic and cross-linked keratoconic eyes. **Material and Methods:** The subjects eligible for the study were composed of two groups: Keratoconus group and Collagen-cross-linking (CXL) group. All subjects underwent three consecutive corneal topography measurements using the Scheimpflug camera (Pentacam, Oculus, Germany). The time between each scan was, typically, about 1-2 min. The repeatability limits of the 3 repeated measurements were calculated. Reliability was assessed by an intraclass correlation coefficient (ICC). **Results:** This study comprised 36 eyes of 18 keratoconus patients and 28 eyes of 14 keratoconus patients cross-linking applied. All ICC values in both groups are more than 0.95. The repeatability limits are higher in CXL group than keratoconus group for all parameters except Q value, root mean square (RMS) of total and horizontal coma. The repeatability limits of K1, K2, Kmax and the thinnest corneal thickness (TCT) were 0.58 D, 0.64 D, 1.77 D and 14.3 µm in keratoconus group, respectively. The repeatability limits of K1, K2, Kmax and TCT were 1.39 D, 1.13 D, 2.06 D and 15.3 µm in CXL group, respectively. **Conclusion:** The higher repeatability limits of K1, K2 and Kmax in CXL group were interesting. These results show the need for studies with larger numbers evaluating the repeatability of Pentacam in keratoconus patients after cross-linking.

ÖZET Amaç: Bu çalışmanın amacı Pentacam ölçümlerinin çapraz bağlama uygulanmış ve uygulanmamış keratokonus hastalarında tekrarlanabilirlik ve güvenilirliğini değerlendirmektir. **Gereç ve Yöntemler:** Çalışmaya dahil edilen kişiler iki gruptan oluştu: Keratokonus grubu, Kollajen çapraz bağlama (KÇB) grubu. Çalışmaya dahil edilen tüm kişilere Scheimpflug kamera sistemi (Pentacam, Oculus, Almanya) ile ardışık üç kere korneal topografi ölçümü yapıldı. Her ölçüm arasında 1-2 dakika süre beklendi. Üç ölçümün tekrarlanabilirlik limiti ölçümlerin %95'inin içinde olduğu varsayılan $1,96 \cdot \sqrt{2}Sw$ olarak hesaplandı. Güvenilirlik sınıfı içi korelasyon katsayısı (SKK) ile değerlendirildi. **Bulgular:** Bu çalışma 18 keratokonus hastasının 36 gözünü ve 14 KÇB uygulanmış keratokonus hastasının 28 gözünü kapsamaktadır. Tüm ölçümler için SKK değerleri üç grupta da 0,95'in üzerindedir. Q, total ve horizontal komanın root mean square (RMS) değerleri haricinde tüm ölçümlerin tekrarlanabilirlik limitleri KÇB grubunda keratokonus grubundan yüksekti. K1, K2, Kmax ve en ince kornea kalınlığı (EİKK) ölçümlerinin tekrarlanabilirlik limitleri keratokonus grubunda sırasıyla 0,58 D, 0,64 D, 1,77 D ve 14,3 µm idi. K1, K2, Kmax ve EİKK ölçümlerinin tekrarlanabilirlik limitleri KÇB grubunda sırasıyla 1,39 D, 1,13 D, 2,06 D ve 15,3 µm idi. **Sonuç:** KÇB grubunda daha yüksek tekrarlanabilirlik limitleri ile birlikte bütün gruplarda Pentacam ölçümlerinde çok iyi düzeyde güvenilirlik gözlemlendi. KÇB grubunda K1, K2 ve Kmax ölçümlerinin daha yüksek tekrarlanabilirlik limitleri dikkat çekiciydi. Bu sonuçlar KÇB sonrası keratokonus hastalarında Pentacam ölçümlerinin tekrarlanabilirliğini değerlendiren daha çok hasta sayılı çalışmalara ihtiyaç olduğunu göstermiştir.

Keywords: Keratoconus; Pentacam; cross-linking; reliability; repeatability

Anahtar Kelimeler: Keratokonus; Pentacam; çapraz bağlama; güvenilirlik; tekrarlanabilirlik

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Scheimpflug imaging devices, such as the Pentacam, are frequently used devices in the management of keratoconus.¹⁻³ These systems work with a rotating Scheimpflug camera. The measurement of corneal elevations, corneal thickness from central to the periphery are possible via these systems. Also anterior and posterior corneal aberrations could be assessed by the calculation of Zernike coefficients. The repeatability of the measurements taken with Pentacam from normal and keratoconic eyes was assessed as good to excellent in the previous studies.^{4,5} The intraclass correlation coefficient (ICC) values for all parameters were between 0.75 and 1.0. Eguileor et al. assessed repeatability and reliability of measurements handled with Scheimpflug system to monitor progression in keratoconus patients and healthy volunteers. Although they found high repeatability for all parameters with high ICC values, they mentioned that none of the parameters met the criteria to ascertain that they were completely reliable for progression monitoring because of calculated relatively high repeatability limits for all parameters.⁴ They did not involve keratoconus patients to whom cross-linking (CXL) was applied.

Kosekahya et al. and Sideroudi et al. researched reliability of Pentacam measurements in healthy volunteers and keratoconus patients. They observed differences between ICC values of normal and keratoconic eyes almost for all measurements.^{5,6} Sideroudi et al. indicated the differences in ICC, within subject standard deviation (Sw), repeatability coefficient of variation (CoV) values between keratoconic and keratoconic eyes that cross linking was performed.⁷ CXL has become an accepted treatment modality effective in stopping progression in keratoconus. It provides new covalent bond formation between collagen fibers by exciting riboflavin molecules with ultraviolet A. This procedure changes corneal structure.

Jin et al. reported decreased agreement between devices in keratometry measurements following corneal refractive surgery.⁶ The conclusion of the probable effect of procedures changing corneal structure on reliability of devices imaging anterior and posterior corneal surface could be sourced from this study. The observed difference in the above men-

tioned study between ICC values of keratoconic and cross-linked keratoconic eyes also supports this opinion.⁴ Vianna et al. had already reported that Pentacam measurements showed increased variability correlated with increased topographic astigmatism in keratoconus patients.⁸

The careful monitoring of progression in keratoconus patient is so essential. In some studies, various topographic criteria were mentioned to determine progression.⁹⁻¹¹ Currently, the same criteria have been used to monitor progression in cross-linked keratoconic eyes. To our knowledge, there is no study assessing repeatability limits of parameters handled with Scheimpflug system to monitor progression in cross-linked keratoconic eyes. The different repeatability limits in CXL applied keratoconus patients could be observed related to the change in corneal shape and haze formation caused by CXL procedure. The aim of this study to evaluate whether CXL changes the repeatability limits of Pentacam measurements in keratoconic eyes or not.

MATERIAL AND METHODS

This was a retrospective study. The medical records of keratoconus patients followed at the Ophthalmology Department of our hospital were analysed. The patients who had at least one session of three consecutive Pentacam measurements were involved in the study. This research was managed in accordance with the tenets of Helsinki Declaration. The study was approved by the Erciyes University Clinical Research Institutional Board (2019/484).

STUDY POPULATION

The patients whose medical records were involved in the study were composed of two groups:

1. Keratoconus group: This group included keratoconus patients with stable measurements (progression has not been observed).
2. CXL group: The keratoconus patients with uneventful corneal collagen cross-linking history (The CXL procedure was performed at least six months prior to the inclusion in the study).

Current or previous corneal diseases such as keratitis, hydrops or corneal scarring, severe dry eye

were exclusion criteria for both groups. Also, the patients with a previous ocular surgery, other ocular diseases such as cataract, glaucoma or galucoma suspect, the history of contact lense use, systemic or ocular drug use, any systemic disorder including autoimmune disease, pregnancy or nursing were excluded from the study. The patients with keratoconus were graded with Amsler Krumeich classification.

CORNEAL COLLAGEN CROSS LINKING PROCEDURE

All procedures were performed under topical anesthesia. The epithelium was detached from the stroma by using a spatula following the application of 20% alcohol for ten seconds. The cornea was exposed to 20% dextran-1% riboflavin solution (Ricrolin, Sooft, Montegiorgio, Italy) during 30 minutes. 9.0 mW/cm² ultraviolet A irradiation (Apollon Crosslinking System, Meram Medicine, Turkey) was applied for 10 minutes (with a distance of 5 cm from cornea). During irradiation, riboflavin application was continued. The operation was ended with the contact lens application. 0.3% ofloxacin (Okacin, Novartis), 0.1% fluorometalon (Flarex, Alcon) and artificial tears were prescribed postoperatively.

PENTACAM SCHEIMPFLUG CAMERA MEASUREMENTS

The standard automated method was used to handle the Pentacam measurements. The same operator obtained all measurements.¹² Three consecutive measurements were executed for all patients. A few minutes interval was given to the patients after each measurement. The following parameters from Pentacam were analysed: minimum (K1) and maximum (K2) keratometry values in the central zone, maximum keratometry across the entire area scanned (Kmax), axis of corneal astigmatism, anterior and posterior best-fit spheres (BFS) in the float mode with the diameter set to 8 mm, maximum anterior and posterior elevations, TCT, and corneal asphericity at 6 mm, root median square (RMS) of the higher order aberrations, vertical and horizontal coma, and spherical aberration.

STATISTICAL ANALYSIS

The measurements were analyzed with SPSS software (version 13.0, SPSS, Inc.). The Shapiro-Wilk W test was used for assessment of data distribution.

The Fisher's exact test was used for the comparison of categorical variables. The categorical variables were expressed as number and percentage for each group separately. Comparison of the stage of keratoconus and age between two groups was performed by the Mann-Whitney U test. The nonparametric variables were expressed as median (25th/75th percentiles). The parametric variables were expressed as mean \pm standard deviation (SD).

The repeatability limit of the repeated measurements was reported as $1.96 * \sqrt{2}Sw$.¹³⁻¹⁵ The within-subject standard deviation (Sw) was standard deviation of 3 consecutive measurements. Reliability was assessed by ICC value. It changes between 0 and 1. The ICC values were assessed according to the study of Kuu TK and Li MY.¹⁶

RESULTS

This study comprised 36 eyes of 18 keratoconus patients and 28 eyes of 14 keratoconus patients cross-linking applied. There is no significant difference in age, gender and stage of keratoconus between two groups ($p=0.410, 0.552$ and 0.223 , respectively). The median age was 28 years (21-33 years) in keratoconus group and 27 years (24-28 years) in CXL group. The median stage was 2 (1-3) in keratoconus group and 2 (2-3) in CXL group. The male to female ratio was 10 (55%) / 8 (45%) in keratoconus group and 8 (57%) / 6 (43%) in CXL group. [Table 1](#) shows the mean values of all measurements with normal distribution and median values of measurements which has not normal distribution. The ICC values together with calculated repeatability limits are listed in [Table 2](#).

All ICC values in both groups were more than 0.95. This result shows excellent reliability for all measurements taken with Pentacam in keratoconus patients and CXL-applied keratoconus patients.

The repeatability limits are higher in CXL group than keratoconus group for all parameters except Q value, RMS of total and horizontal coma. The repeatability limits of K1, K2, Kmax and TCT were 0.58 D, 0.64 D, 1.77 D and 14.3 μ m in keratoconus group, respectively. The repeatability limits of K1, K2, Kmax and TCT were 1.39 D, 1.13 D, 2.06 D and 15.3 μ m in CXL group, respectively.

TABLE 1: The mean and median values of the parameters for both groups.

Parameter (Mean±SD)	Keratoconus Group (n= 36 eyes)	Cross-linking Group (n= 28 eyes)
	K1,D	45.44±2.78
K2,D	48.19±3.76	50.46±4.10
Kmax,D	52.06±5.37	55.2±6.10
Ast	2.85±1.92	4.55±2.31
BFS_ant, mm	7.53±0.32	7.41±0.29
BFS_post, mm	6.12±0.28	6.03±0.25
TCT, µm	452±19	444±4
Q	-0.68±0.4	-0.89±0.23
RMS Total	6.61±3.57	8.28±3.35
RMS HOA	1.65±1	2.06±0.93
Z ₃ ⁻¹ , µm *	-0.02 (-0.32 / 0.38)	-0.16 (-0.70 -0.72)
Z ₃ ¹ , µm *	-1.13 (-1.97 / -0.63)	-1.13 (-1.52 / -0.73)
Z ₄ ⁰ , µm *	-0.17 (-0.56 / 0.15)	-0.41 (-0.82 / -0.01)

Z₃⁻¹: Horizontal coma, Z₃¹: Vertical coma, Z₄⁰: Spherical aberration

*: median values (25th / 75th percentiles).

SD: Standard deviation, BFS: Best-fit spheres, TCT: Thinnest corneal thickness,

RMS: Root mean square, K1: Minimum keratometry value,

K2: Maximum keratometry value.

TABLE 2: The repeatability limits and ICC values of all parameters for both.

Parameter	Keratoconus Group (n= 36 eyes)		Cross-linking Group (n= 28 eyes)	
	Repeatability limit	ICC; P value	Repeatability limit	ICC; P value
	K1,D	0.58	0.99; <0.001	1.39
K2,D	0.64	0.99; <0.001	1.13	0.90; <0.001
Kmax,D	1.77	0.99; <0.001	2.06	0.99; <0.001
Ast	0.76	0.99; <0.001	1.06	0.99; <0.001
BFS_ant, mm	0.22	0.98; <0.001	0.27	0.96; <0.001
BFS_post, mm	0.08	0.99; <0.001	0.14	0.95; <0.001
TCT, µm	14.3	0.99; <0.001	15.3	0.98; <0.001
Q	0.25	0.98; <0.001	0.14	0.98; <0.001
RMS Total	1.19	0.99; <0.001	0.86	0.99; <0.001
RMS HOA	0.21	0.95; <0.001	0.39	0.99; <0.001
Z ₃ ⁻¹ , µm	0.28	0.95; <0.001	0.58	0.98; <0.001
Z ₃ ¹ , µm	0.55	0.98; <0.001	0.40	0.96; <0.001
Z ₄ ⁰ , µm	0.32	0.97; <0.001	0.51	0.96; <0.001

Z₃⁻¹: Horizontal coma, Z₃¹: Vertical coma, Z₄⁰: Spherical aberration.

SD: Standard deviation, BFS: Best-fit spheres, TCT: Thinnest corneal thickness,

RMS: Root mean square, K1: Minimum keratometry value,

K2: Maximum keratometry value.

Figure 1, Figure 2 and Figure 3 showed three consecutive measurements of a patient from keratoconus group. The differences in Kmax between consecutive measurements were noteworthy.

DISCUSSION

The reliability and repeatability of Pentacam in eyes with keratoconus with the history of CXL application were assessed in this study. It was aimed to establish possible differences sourced from the changed structure of cross-linked cornea in reliability and repeatability of Pentacam between keratonic eyes and cross-linked keratoconic eyes. Jin et al. showed that the structural changes on cornea could affect the reliability and repeatability of machines.⁶ They have reported that myopic laser refractive surgery changed the agreement between Pentacam, Orbscan and IOLMaster in measurements. While 95% limits of agreement between Pentacam, Orbscan and IOLMaster before laser surgery were 1.31 D, 0.79 D and 1.14 D, LOAs after the laser surgery were 1.47 D, 1.14 D, and 1.34 D, respectively.

In this study, the measurements of keratoconus patients regardless of being applied CXL with Pentacam showed excellent reliability for all parameters. The repeatability limits of measurements were higher in CXL group than keratoconus group. Especially, the repeatability limits of K2, Kmax and TCT (0.64 D, 1.77 D and 14.3 µm in keratoconus group; 1.13 D, 2.06 D and 15.3 µm in CXL group, respectively) were interesting. Because these three parameters were so important for the detection of progression.

Keratoconus is a non-inflammatory progressive ectatic disease that could result in impairment in visual acuity. The spectacles, contact lense and some surgical interventions like intracorneal ring implantation or corneal transplantation can improve visual acuity in keratoconus patients but the only modality halting progression of the disease is corneal CXL and the detection of progression has such importance as to decide CXL application and to assess the success of CXL. Thus, reliable criteria are essential to establish an indication for CXL and to follow-up after CXL. The Global Consensus on Keratoconus and Ectatic Diseases describes progression as increase in the anterior and posterior corneal surface elevation, decrease

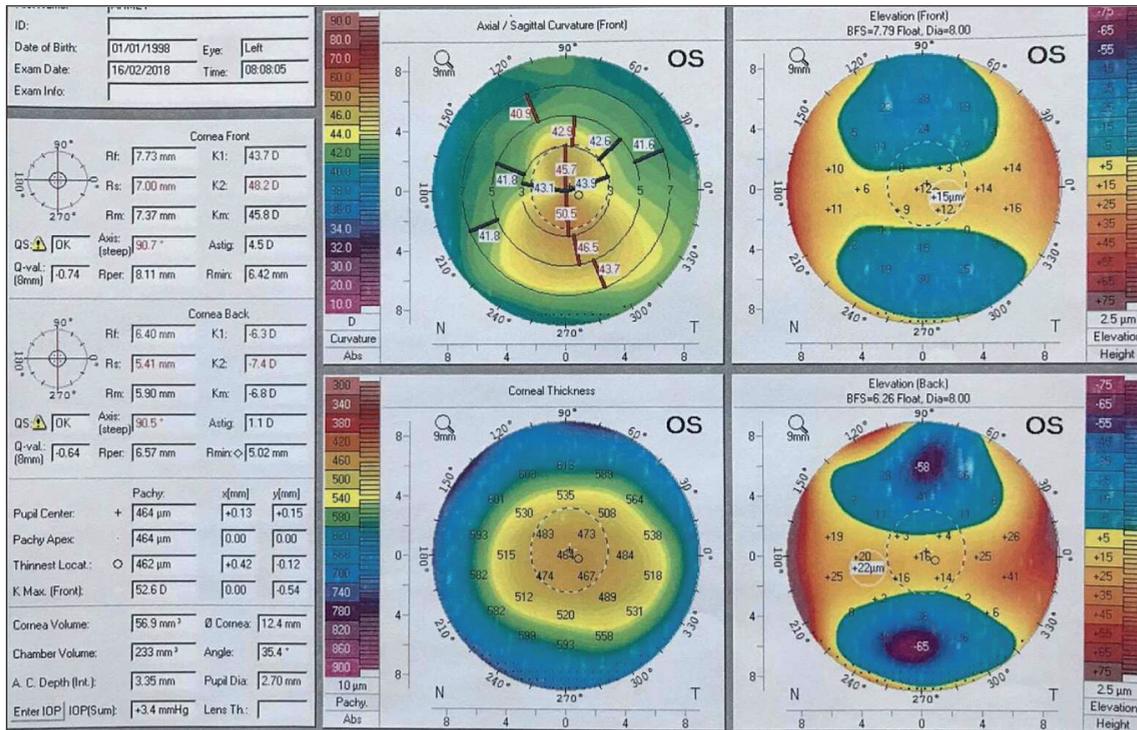


FIGURE 1: The first measurement of a patient in keratoconus group.

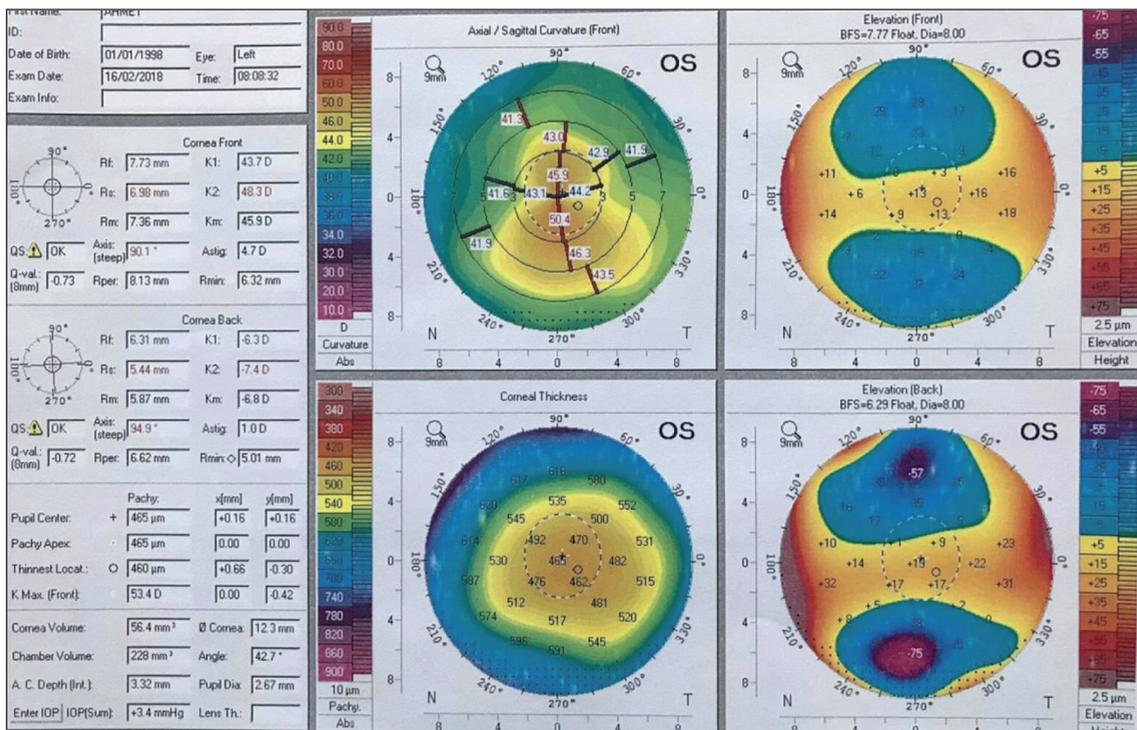


FIGURE 2: The second measurement of a patient in keratoconus group.

in corneal thickness, and/or an increase in the amount of the corneal thickness decrease between the periphery and the thinnest corneal point, but this definition

does not contain the values related to amount of progression.¹⁷ Several studies have been conducted to establish criteria for progression.⁹⁻¹¹ The criteria from

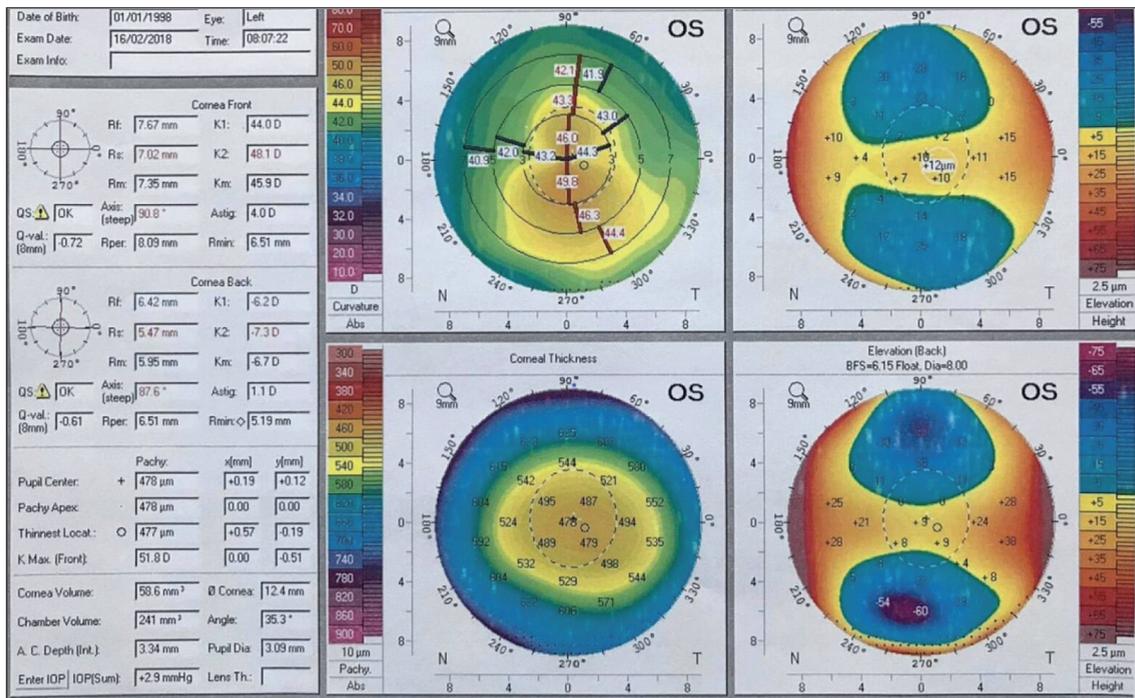


FIGURE 3: The third measurement of a patient in keratoconus group.

the studies include: 1D increase in Kmax during 1 year, 1-1.5 D increase in K2 during 6 months, 10 µm thinning in the thinnest point, 20 µm thinning in the thinnest point, 5% decrease in the thinnest corneal thickness. The repeatability limits of Kmax and TCT in our study were higher from the limits of almost all of these studies. As a result, the criteria for the detection of progression remained incompetent especially in keratoconus patients to whom CXL was applied.

The Oculus Pentacam (Oculus, Wetzlar, Germany) is an anterior segment topographer. It was reported that Pentacam had good to excellent reliability and repeatability in keratoconus patients. From recent studies: Kosekahya et al. assessed the repeatability and reliability of Pentacam measurements in eyes with keratoconus and they found good to excellent repeatability and reliability for all parameters.⁵ Sideroudi et al. established that simulated keratometry, coma, coma-like and higher-order RMS have enough repeatability, reliability and reproducibility to assess posterior corneal surface in keratoconic and cross-linked keratoconic eyes.⁷ They also reported that other parameters apart from these showed limited repeatability but they did not focus on repeatability limits of the measurements. Eguileor et al. focused on

the reliability and repeatability of parameters handled with Pentacam to detect progression in keratoconus.⁴ They analysed K1 and K2 values, the axis of corneal astigmatism, anterior and posterior BFS, the maximum anterior and posterior elevations, TCT, corneal asphericity at 6 mm, RMS of the higher order aberrations, RMS of third-order coma, coma axis, vertical and horizontal coma, and spherical aberration. They concluded that Pentacam has good to excellent reliability for all parameters. However, it showed some limitations in the use for progression analysis related to repeatability limits of 1.54 D for the maximum keratometry, 1.08 D for K2 value, 14.2 µm for thinnest corneal thickness. These results were compatible with our results. But they did not assess the repeatability limits of Pentacam in keratoconic eyes CXL applied.

This study has some limitations. First, the number of patients involved in the study was low. The studies with large number of patients could establish precise repeatability limits. Second, inter-session or inter-observer reliability and repeatability of measurements were not assessed. Last, we did not involve healthy volunteers in this study. The comparison of repeatability limits of keratoconus patients with healthy people could be informative.

In conclusion, it was found that there was excellent reliability in both groups with higher repeatability limits in CXL group than keratoconus group. Especially, the repeatability limits of K2 and Kmax in both groups are interesting. The higher repeatability limits in CXL group might be a consequence of structurally changed structure of cornea or Pentacam technology. To date, this is the first study assessing repeatability limits of Pentacam in cross-linked keratoconic eyes. The studies with other machines assessing cornea for their reliability and repeatability in cross-linked eyes are essential. These results show the need for studies with larger numbers to establish the precise limits of repeatability of Pentacam in keratoconus patients before and after CXL. It could be possible to construct the reliable criteria to monitor the progression in keratoconus patients only with precise repeatability limits of machines measuring corneal parameters.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Ayşe Çiçek, Hayrettin Çobanoğlu; **Design:** Ayşe Çiçek, Mahmut Erkam Arslan; **Control/Supervision:** Ayşe Çiçek, Esra Vural; **Data Collection and/or Processing:** Ayşe Çiçek, Mustafa Ataş; **Analysis and/or Interpretation:** Ayşe Çiçek, Mustafa Ataş, Mahmut Erkam Arslan, Hayrettin Çobanoğlu; **Literature Review:** Ayşe Çiçek, Mustafa Ataş; **Writing the Article:** Ayşe Çiçek, Mahmut Erkam Arslan, Bedirhan Alabay; **Critical Review:** Ayşe Çiçek, Mustafa Ataş; **References and Fundings:** Ayşe Çiçek, Mustafa Ataş, Esra Vural; **Materials:** Ayşe Çiçek, Mustafa Ataş, Bedirhan Alabay.

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