ARTICLE

Corneal Densitometry Changes in Emmetropic Presbyopic Eyes Three Years After Sterile Allograft Corneal Lenticle Implantation

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Purpose: To evaluate corneal densitometry changes in the eyes 3 years after allograft corneal inlay (ACI) implantation for refractive treatment of presbyopia.

Methods: This retrospective case series included 32 nondominant eyes of 32 patients who underwent allograft corneal lenticle implantation. Corneal densitometry was performed preoperatively and postoperatively at 1, 3, 6, 12, and 36 months using a Scheimpflug tomography device. The results were analyzed in four different layers and four different zones of the cornea.

Results: The mean age of the 32 included patients was 49.8±3.3 (range 45–56) years. The densitometric changes measured at 36 months were more prominent in zones 1 and 2, especially in the anterior layer (AL). The AL mean baseline densitometry values were 19.02±2.20 grayscale unit (GSU) for zone 1 and 17.50±1.89 for zone 2. Densitometry values increased significantly at 1-month (to zone 1:23.37±3.71 GSU, $P<0.001$ and zone 2:18.90±2.39 GSU, $P=0.001$) and 3-month follow-up (to zone 1:23.68±4.35 GSU, $P<0.001$ and zone 2:18.80±2.46 GSU, $P=0.006$). Between the 3-month and 6-month follow-up periods, the densitometry values returned to baseline levels and remained constant throughout the 36-month follow-up (to zone 1:19.98±1.96 GSU, $P=0.414$ and zone 2:18.11±2.13 GSU, $P=1.000$). No significant densitometric changes were observed in zone 3 at any time. Densitometric values returned to baseline levels in all layers and zones at 6 months postoperatively.

Conclusion: These results support the notion that ACI implantation is relatively safe regarding corneal transparency.

Key Words: Allograft corneal inlay—Presbyopia—Corneal densitometry—Corneal lenticule.

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Synthetic corneal inlays have been reported to provide satisfactory near vision acuity results as an alternative to intraocular surgery in the treatment of presbyopia.[1] In addition, the absence of laser tissue removal during inlay implantation makes the procedure superior to laser ablation treatments. Today, there are biocompatible, nonallogeneic, synthetic corneal inlays, and allogeneic corneal inlay options. However, serious side effects, such as decreased visual acuity, infectious keratitis, inlay decentralization, and epithelial ingrowth, have been reported after synthetic corneal inlay implantation.[2–5] Another reported side effect is the development of corneal haze, which may cause a decrease in visual acuity.[6,7]

The transparency of the cornea is essential for good visual function. For the cornea to remain transparent, the endothelial and stroma layers must be healthy and work in harmony.[8,9] The transparency of the cornea may decrease after corneal infection, ectasia, dystrophy, corneal edema, or corneal surgery. Many methods are used to evaluate the transparency of the cornea. Slitlamp examination and imaging of the cornea are among the commonly used methods. Hanna et al.[10] scored the development of clinical haze after corneal surgery subjectively in their studies. Apart from this, there are some methods that objectively score haze and scar formation after corneal surgery.[11–14]

However, quantitative, reproducible clinical measurement methods are needed to objectively grade and monitor corneal transparency. In recent years, measurement of corneal densitometry (CD) with Scheimpflug (Pentacam, Oculus, Wetzlar, Germany)[15] imaging has become a frequently used method to quantitatively evaluate corneal haze development after corneal refractive surgeries. This objective way of determining the light backscattered by the cornea, termed CD, has been used to analyze CD after corneal crosslinking, corneal ring implantation, photorefractive keratectomy (PRK), laser in situ keratomileusis (LASIK), and small incision lenticule extraction (SMILE).[15–19]

The use of allograft corneal lenticles in refractive surgery is not a new method. In fact, there are several studies presenting allograft corneal lenticle implantation, especially in the treatment of hyperopia.[20–23] In a previous study, we presented the initial refractive results of allograft corneal lenticles used in the treatment of presbyopia.[24] However, we did not report early and long-term densitometric results at that time.

The aim of this study was to perform objective and subjective evaluation of corneal clarity through densitometry and slitlamp microscopy over a 36-month follow-up period after presbyopic allograft corneal inlay (ACI) implantation. To the best of our knowledge, there is no other study in the literature addressing densitometric changes in the cornea after allograft corneal lenticle implantation for the treatment of presbyopia.

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Ethics committee approval was received from the Istanbul Medipol University Ethics Committee on 21/05/2020 with the number E-10840098-604.01.01-E.15487 for this study.

Informed consent form was taken from all patients for this study.

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MATERIALS AND METHOD

In this retrospective, case series study, patients who had ACI (Transform, Allotex Inc Boston, MA) implanted in their non-dominant eye for presbyopia were included. All implantations were performed between April 2018 and October 2019. The study was conducted in accordance with the principles of the Declaration of Helsinki and with the approval of the Istanbul Medipol University Institutional Review Board/Ethics Committee (21.05.2020, 10840098-604.01.01-E.15487, Approval Number 407). Informed consent was obtained from all patients before surgery in accordance with legal requirements.

The inclusion criteria for the treated eye were stable refraction (manifest refractive spherical equivalent (MRSE) change of less than 0.50 D in the past 12 months), MRSE between −0.75 D and +1.00 D, and cylindrical refraction less than 0.75 D. Wearers of rigid gas-permeable contact lenses were asked to discontinue use of their contact lenses at least 3 weeks before the first examination, and wearers of soft contact lenses at least 2 weeks before the first examination. The exclusion criteria were history of ocular disease or ophthalmic surgery in the treated eye, presence of keratoconus or other ecstatic corneal disease, central corneal thickness less than 500 μm, history of herpetic infection, collagen tissue disease, pregnancy, or breastfeeding.

Corneal densitometry was evaluated with Scheimpflug tomography (Pentacam; Oculus Inc, Wetzlar, Germany) at baseline and at the 1st, 3rd, 6th, 12th, and 36th months postoperatively. In this study, CD of zones of different diameters and different depths of the cornea was analyzed using the mean densitometry table automatically calculated by Pentacam (version 1.20r36). The 10-mm diameter of the cornea was divided into four radial regions. These annular zones were zone 1: 0 to 2 mm, zone 2: 2 to 6 mm, zone 3: 6 to 10 mm, and whole cornea: 0 to 10 mm. In addition, each corneal zone was evaluated in four layers based on depth: the anterior layer (AL) (anterior 120 μm, AL), the posterior layer (PL) (posterior 60 μm, PL), the central layer (CL; between the AL and PL), and the total cornea (Fig. 1). Subjective evaluation of ACI implantation was performed by slitlamp microscopy. At all postoperative follow-ups, patients were checked under slitlamp microscopy for possible side effects and complications such as graft rejection, corneal haze, dry eye, infection, and inflammation. We did not use a scoring system in subjective evaluation.

Surgical Technique

Under sterile conditions, proparacaine HCl 0.5% (Alcaine, Alcon, Fort Worth, TX) was instilled for topical anesthesia. As a first step, a femtosecond laser-assisted (iFS 150 kH, Intralase, Johnson & Johnson Vision, ABD) 70-degree superior-hinged flap with a thickness of 110 μm and a diameter of 8.8 mm was created. The stromal interface was washed with balanced salt solution during and after flap opening. As a second step, the ACI was placed in the center of the pupil with the help of a specially designed stainless steel loop apparatus under the microscope. Finally, the flap was closed with the help of a cannula, making sure that the edges of the corneal inlay were regular and in the center of the pupil, and a soft bandage contact lens (Bausch & Lomb PureVision TM, Rochester, NY) was applied. All ACI implantations were performed by the same surgeon (A.K.). The allograft corneal inlays used in the study were 2.8 mm in diameter and approximately 25 μm thick, with a target refractive power of +2.50 D. All ACIs used in this case series were screened and processed according to US eye banking regulation and then shaped by the manufacturer (Allotex Inc Boston, MA) with an excimer laser. Sterile ACIs come in a solution in a ready-to-use bottle with a shelf life of up to 2 years, which can be stored at room temperature. Postoperatively, topical moxifloxacin 0.5% (Vigamox, Alcon Laboratories, Fort Worth, TX) was applied five times a day for 3 weeks postoperatively. Preservative-free artificial tears (Refresh; Allergan, Inc, Irvine, CA) and topical dexamethasone 0.1% (Maxidex; Alcon Laboratories Inc, Fort Worth, TX) were prescribed five times daily for 6 weeks.

Statistical Analysis

SPSS Statistics for Mac version 25.0 (IBM Corp., Armonk, NY) was used for statistical analysis. The Shapiro–Wilk test and box plot graphs were used to evaluate whether the variables were normally distributed. Repeated-measures ANOVA and Bonferroni tests were used to analyze repeated measures of normally distributed variables at baseline and postoperative 1-, 3-, 6-, 12-, and 36-month follow-ups. The Friedman test and Wilcoxon signed-rank test were used in the analysis of repeated measures that did not show a normal distribution. A *P* value below 0.01 was considered significant after Bonferroni correction.

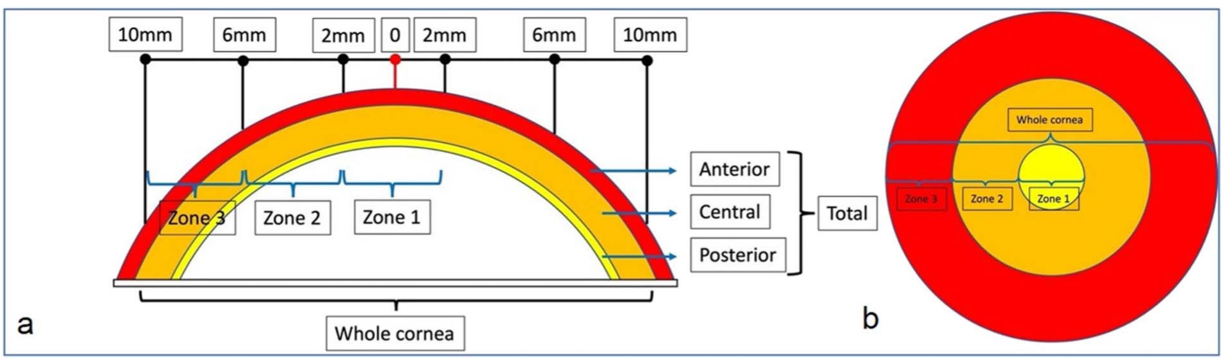

FIG. 1. Schematic drawing of optical CD analysis with Pentacam. (A) Optical CD of annular zones (zone 1: 0–2 mm, zone 2: 2–6 mm, zone 3: 6–10 mm); (B) optical CD based on depth (AL: Superficial 120 μm, CL: between the anterior and posterior layers, PL: Deep 60 μm). AL, anterior layer; CD, corneal densitometry; PL, posterior layer.

Table 1. *Pre- and Postoperative CD Changes in Presbyopic Patients With Sterile Allograft Corneal Lenticle Implanted*

			Postoperative Mean±SD					
Zone	Layers	Preoperative (1) Mean±SD	1 Month (2)	3 Months (3)	6 Months (4)	12 Months (5)	36 Months (6)	*P*
0–2 mm	Anterior	19.02±2.20	23.37±3.71	23.68±4.35	19.98±3.66	19.05±3.24	19.98±1.96	<0.001*
		(1) vs. (2) post hoc *P*=0.000*; (1) vs. (3) post hoc *P*=0.000*; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.414						
	Central	14.80±1.22	15.68±1.57	15.88±1.64	14.58±1.82	14.42±1.52	15.03±1.02	<0.001*
		(1) vs. (2) post hoc *P*=0.002*; (1) vs. (3) post hoc *P*=0.000*; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=1.000						
	Posterior	11.74±1.08	11.57±1.11	11.74±1.19	11.21±1.37	11.20±1.11	11.83±1.23	0.01
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=0.773; (1) vs. (5) post hoc *P*=0.374; (1) vs. (6) post hoc *P*=1.000						
	Total	15.20±1.36	16.87±2.00	17.15±2.22	15.25±2.20	14.88±1.81	15.62±1.08	<0.001*
		(1) vs. (2) post hoc *P*=0.000*; (1) vs. (3) post hoc *P*=0.000*; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.519						
2–6 mm	Anterior	17.50±1.89	18.90±2.39	18.80±2.46	17.15±3.13	16.65±2.68	18.11±2.13	<0.001*
		(1) vs. (2) post hoc *P*=0.001*; (1) vs. (3) post hoc *P*=0.006*; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=1.000						
	Central	13.76±1.31	13.90±1.57	13.95±1.50	13.20±1.96	13.13±1.72	13.93±1.45	0.002*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=0.501; (1) vs. (6) post hoc *P*=1.000						
	Posterior	10.99±1.01	10.71±1.19	10.75±1.07	10.37±1.39	10.40±1.21	11.07±1.23	0.001*
		(1) vs. (2) post hoc *P*=0.335; (1) vs. (3) post hoc *P*=0.608; (1) vs. (4) post hoc *P*=0.137; (1) vs. (5) post hoc *P*=0.089; (1) vs. (6) post hoc *P*=0.335						
	Total	14.08±1.33	14.51±1.63	14.54±1.57	13.57±2.11	13.38±1.79	14.38±1.43	<0.001*
		(1) vs. (2) post hoc *P*=0.341; (1) vs. (3) post hoc *P*=0.214; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=0.480; (1) vs. (6) post hoc *P*=1.000						
6–10 mm	Anterior	21.84±6.76	22.58±6.35	22.48±6.25	21.61±7.58	21.02±6.72	23.48±7.46	0.001*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.153						
	Central	19.12±6.24	19.40±6.40	19.20±6.23	19.13±7.04	18.67±6.53	20.31±6.95	0.054
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.290						
	Posterior	15.16±4.01	15.09±4.39	14.96±4.10	14.66±4.32	14.66±4.32	15.98±4.58	0.001*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.311						
	Total	18.71±5.63	19.04±5.66	18.95±5.48	18.33±6.29	18.12±5.81	19.92±6.27	0.001*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.193						
Total	Anterior	20.85±3.45	22.20±3.66	22.28±3.92	20.94±4.86	20.30±3.98	21.95±4.02	0.001*
		(1) vs. (2) post hoc *P*=0.032*; (1) vs. (3) post hoc *P*=0.067; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.208						
	Central	17.30±2.99	17.51±3.26	17.58±3.25	17.05±3.83	16.86±3.35	17.91±3.45	0.058
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.931						
	Posterior	13.80±2.10	13.58±2.40	13.58±2.27	13.28±2.54	13.21±2.34	14.25±2.48	0.001*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.365						
	Corneal	17.31±2.78	17.76±3.04	17.81±3.04	17.08±3.69	16.79±3.13	18.04±3.24	0.007*
		(1) vs. (2) post hoc *P*=1.000; (1) vs. (3) post hoc *P*=1.000; (1) vs. (4) post hoc *P*=1.000; (1) vs. (5) post hoc *P*=1.000; (1) vs. (6) post hoc *P*=0.383						

CD, corneal densitometry.

RESULTS

Thirty-two eyes of 32 patients with presbyopia were included in the study. The mean age of the cases was 49.8±3.3 (range 45–56) years. Of the 32 eyes that underwent surgery, 10 were right and 22 were left eyes. Twenty-four (75%) of the patients were male, and 8 (25%) were female. All allograft corneal lenticle implantations were accomplished with no intraoperative complications, and no major postoperative complications were seen.

Table 1 summarizes the mean preoperative and postoperative CD parameters and the post hoc analysis. When we analyzed the results of the study after ACI implantation, it was seen that zone 1 CD values increased in all layers except the PL in the 1st month ($P<0.01$), tended to decrease until the 3rd month, but remained above the normal limits ($P<0.01$) and regressed to within normal limits in the postoperative 6th month ($P>0.01$). In zone 2, it was observed that only anterior CD increased in the 1st month ($P<0.01$), remained above the normal limits although it regressed until the 3rd month ($P<0.01$), and decreased to within normal limits in the postoperative 6th month. Compared with the baseline, no significant difference was observed in zone 2 CD except in the AL at 1, 3, 6, 12, and 36 months ($P>0.01$ for all). Compared with the baseline, no significant difference was observed in the CD of zone 3 in any layer and at any follow-up time ($P>0.01$ for all). When we analyzed the whole cornea, it was observed that anterior CD values increased only in the 1st month ($P<0.01$) but returned to baseline values in the third postoperative month ($P>0.01$); no significant changes were observed in the other layers ($P>0.01$). Compared with the baseline, no significant difference was observed in the CD of the PL of all zones ($P>0.01$ for all). It was observed that CD values returned to preoperative values in all layers and all zones in the postoperative 6th month ($P>0.01$ for all) and remained stable in the 12th ($P>0.01$ for all) and 36th months ($P>0.01$ for all). Figure 2 shows graphically the changes in anterior and central CD in zone 1 and zone 2, which are particularly affected by ACI implantation, during the 3-year follow-up.

Slitlamp Examination

At the 1-month postoperative examination, the margins of the inlay could be distinguished only in 15 eyes during slitlamp examination. The corneal inlays were invisible in all patients at the 3-month follow-up after surgery. Graft rejection, corneal haze, and scarring were not observed in any of the cases in the 36-month postoperative follow-up (Fig. 3).

DISCUSSION

In this study, the long-term CD and light microscopy findings in patients who had sterile allograft corneal lenticle implanted for the treatment of presbyopia were reported. During the first 3 months, the CD values increased and then gradually decreased until 6 months, at which time the preoperative baseline was reached. This change occurred especially in zones 1 and 2 and was more prominent in the AL and CL. Zone 3 and the PL were not affected in any way by this surgery. When we consider the results of total CD, it is noteworthy that there was only a significant increase in the AL in the 1st month. As a result, 6 months after surgery, corneal transparency decreased to preoperative levels. The results of our study matched our expectations because a standard 110 μm LASIK flap was used in the study and the corneal lenticule was placed under this flap at the center of the pupil. For this reason, the most prominent changes in zone 1 may be related to the graft placed in the pupil area close to the central cornea.

In the past, Kamra and Raindrop corneal inlays were widely used in the treatment of presbyopia. Although early clinical results of these corneal inlays were promising, they were discontinued due to long-term side effects. The development of an inflammatory reaction in the corneal stroma after corneal inlay surgery using synthetic materials is a technically predictable condition. Implanting presbyopia-correcting inlays within the corneal stroma, at the center of the pupil, has the potential to threaten visual acuity.[1] Initial case series reports of KAMRA inlays showed an incidence of corneal haze of approximately 2% 1 to 2 years after surgery.[25] This ratio was found to be 10% to 14% for Raindrop corneal inlay.[26] Histological examination of the removed inlays showed an increase in fibro-connective tissue around the inlay associated with the transformation of keratocytes into myofibroblasts.[27] Fenner et al.[28] examined the proteomic changes associated with the development of corneal haze after Raindrop inlay surgery in patients and reported that they may be associated with complement cascade components, immunoglobulins, and innate immune system signaling molecules as potential mediators. Ultimately, the Food and Drug Administration (FDA) noted that the incidence of corneal haze of any severity for this inlay increased from 16.1% at 2 years postoperatively to 75% (113 of 150 patients) at 5 years, with 2.0% of patients experiencing a loss of two or more lines of vision. Importantly, they recommended that the device be discontinued immediately.[1]

Corneal densitometry is an objective method that gives an idea about the transparency of the cornea. In recent years, measurement of CD has become a frequently used method to determine corneal transparency after refractive surgery.[18,19,29–31] Ni Dhubhghaill et al.[32] published the normative values of CD in a large case series in a healthy population. According to this study, although CD values were similar in the central and 2 to 6 mm zone, densitometric values increased significantly in the 6 to 10 and 10 to 12 mm zones. In addition, anterior CD values were found to be significantly higher than central and posterior corneal ones. Lopes et al.[33] compared the CD values of keratoconus and healthy subjects. In this study, CD values were found to be higher, especially in the central and 2 to 6 mm zones, in cases with keratoconus. Kim et al.[34] reported that CD values of keratoconus patients who underwent crosslinking peaked in the 1st month postoperatively and continued to decrease until the 6th month. In this study, the CD

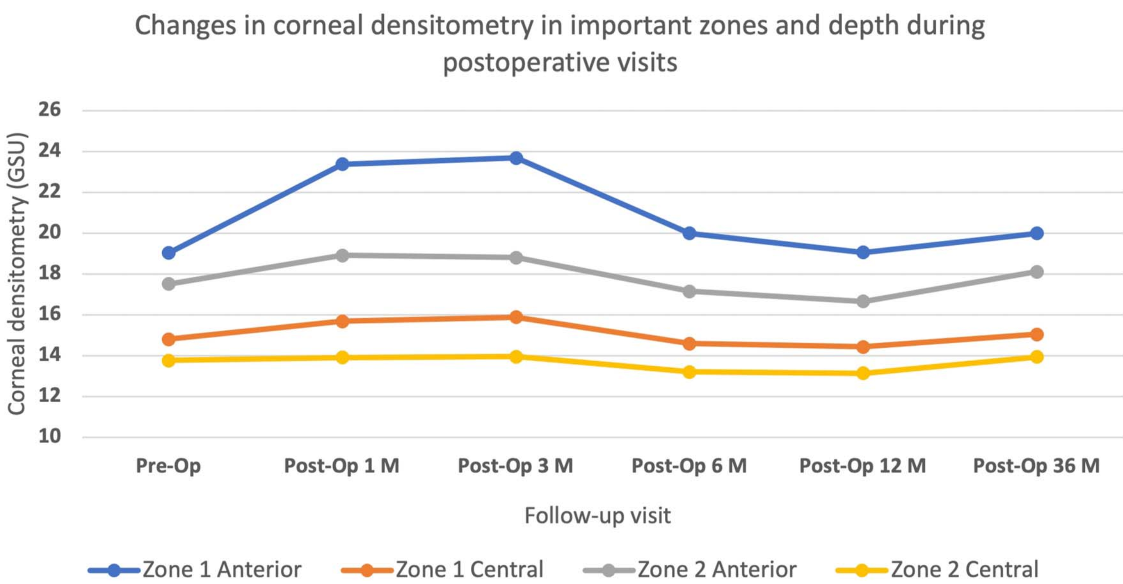

FIG. 2. Changes in anterior and central CD in zones 1 and 2 after ACI implantation. ACI, allograft corneal inlay; CD, corneal densitometry.

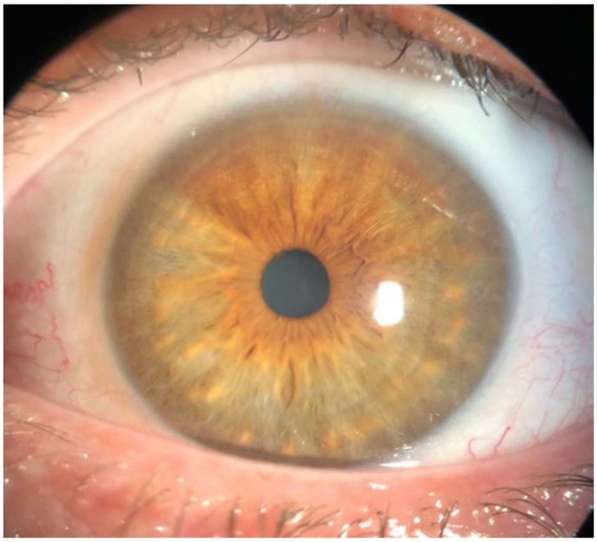

FIG. 3. Slitlamp microscopy view of an eye with implanted sterile allograft corneal lenticle.

measurements of the treated eyes were found to be significantly higher than those of the control eyes, even at the postoperative 6th month. Sedaghat et al.[17] investigated the changes in CD in patients who underwent Keraring corneal inlay implantation in the treatment of keratoconus. In this study, a statistically significant increase was observed in densitometric values in all zones 6 months after the operation, except the 10 to 12 mm annulus of the cornea. The highest increase in CD was observed in the 2 to 6 mm annulus and in the anterior cornea. We believe the increase in CD observed in this study may be related to the synthetic material we used.

Shajari et al.[35] compared the postoperative CD values of patients who underwent Femtosecond Laser-Assisted in Situ Keratomileusis and SMILE. In this study, a small densitometric difference was observed between the two groups in the 1st month, but this difference disappeared at the 3rd month. Cennamo et al.[1] investigated the changes in CD in patients who underwent PRK. In this study, it was reported that CD values were found to be significantly higher in patients with myopia in the range from 6.00 to 12.00 diopters and in those who underwent PRK, even 1 year after the operation, compared with the control group. Piyacomn et al.[36] investigated the postoperative CD change in patients who underwent crosslinking combined with corneal refractive surgery. In the study, it was observed that CD increased in the 0 to 10 mm zone in the anterior cornea and in the 0 to 6 mm zone in the central cornea at one and 3 months. In the 6th month, it was observed that CD decreased to the baseline level in the 0 to 6 mm zone. However, it was reported that densitometric measurements in the AL and CL in the 6 to 10 mm zone remained higher than the baseline level. The main limitation of this study is that the study group was a mixed group including patients who had undergone PRK, LASIK, and SMILE, and densitometric changes were evaluated for the total patient group. Takacs et al.[15] investigated the change in postoperative CD in myopic and hyperopic patients who underwent PRK. They evaluated the operated cases clinically in two groups: those who did and those who did not develop corneal haze. They observed that CD values were significantly higher in cases with haze than in cases without haze. Corneal densitometric measurements of myopic and hyperopic eyes that did not develop haze were found to be like those of patients who did not undergo surgery. In addition, it was observed that CD values decreased significantly from central to peripheral in myopic eyes and from peripheral to central in hyperopic eyes in cases with haze. This study shows that haze development is a very important problem in corneal transparency and haze formation is closely related to the ablation profile. As apparent from the existing literature, any surgical trauma to the cornea can cause changes in CD. However, in surgical methods that can be considered safe, this densitometric change is expected to decrease to normal levels within 3 to 6 months postoperatively. Otherwise, the surgery may have a negative effect on corneal transparency. In their review of allogeneic lenticule implantation, Moshirfar et al.[37] reported that these lenticules can be used in the treatment of corneal ectasia, hyperopia, and presbyopia, and may even be promising in cases of corneal ulcers and perforations.

The limitations of our study are that the results were obtained from a small patient group that did not meet the power analysis and only from our own cases, it did not include a control group, and the subjective evaluation of postoperative corneal transparency was not conducted with a scoring system.

As a result, we observed that although the graft was visible by light microscopy and the densitometric values increased in the 1st months, all effects disappeared over time. In this study, we found the ACI used in the treatment of presbyopia to be quite safe regarding long-term corneal transparency. This is the first study to evaluate the long-term results of the use of allograft corneal inlays in the treatment of presbyopia in corneal transparency. There is a need for studies involving large series of patients to increase the experience on this subject.

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