Original Article

The Surgical Outcomes of Limbal Allograft Transplantation in Eyes Having Limbal Stem Cell Deficiency

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Abstract

Purpose: To report the limbal allograft transplantation and penetrating keratoplasty (PK) results in limbal stem cell deficiency (LSCD)-developed eyes because of chemical or thermal injury.

Methods: Medical records of 18 eyes of 14 patients who had undergone keratolimbal allograft (KLAL) or living-related conjunctival limbal allograft (lr-CLAL) with or without PK and followed up at least 1 year postoperatively were evaluated retrospectively. The preoperative LSCD grade was noted in all patients. Rejection incidents, recurrence of LSCD, and corneal graft clarity along with a visual improvement during the follow-up were noted. The complications rate due to surgery or injury itself, for instance, glaucoma and cataract, were evaluated. The limbal allograft tissue survival analysis and corneal allograft survival analysis were done to reveal the differences in both the procedures. The existence of normal corneal epithelium and improvement in visual acuity were accepted as the surgical success criteria.

Results: In the limbal allograft transplantation group, the survival rates of the allograft tissue were 65 ± 10.7% at 1 year and 36.6 ± 11.4% at 3 years in lr-CLAL and 66.7 ± 15.7% at 12 months and 53.3 ± 17.3% at 18 months in KLAL-transplanted eyes. The survival rate of corneal allograft at the 5th postoperative year was lower in the simultaneous procedure compared to the staged procedure, but it was not statistically significant (25.7 ± 25.8% vs. 62.5 ± 17.1%, $P = 0.75$). The ambulatory vision was achieved in 10 eyes (56%) after a mean follow-up time of 93.8 ± 37.8 months. The visual acuity level has increased in 12 eyes (67%) in which the limbal allograft transplantation was applied. The ambulatory visual acuity level was achieved (≤1.0 logMar [20/200]) in 10 eyes (56%). In addition, two or more Snellen lines' gain in the best corrected visual acuity was observed in 12 eyes of 18 (67%) at the last follow-up, and there was not any significant difference between the KLAL and lr-CLAL.

Conclusions: Ocular surface integrity was longer in KLAL than in lr-CLAL transplantation, but it was not statistically significant. The staged procedure was more convenient than the simultaneous procedure in terms of corneal allograft clarity maintenance in limbal allograft-employed eyes.

Keywords: Allograft limbal transplantation, Chemical injury, Limbal stem cell deficiency, Penetrating keratoplasty

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Introduction

To maintain corneal clarity, the integrity of the corneal epithelium is crucial.[1] The corneal epithelium originates from the limbal stem cells located in the limbus.[1] Limbal stem cells are ultimately responsible for the renewal and regeneration of the corneal epithelium.[2] A decrease in the number of limbal stem cells due to ocular surface injury leads to the corneal opacity and scar development.[3] In such cases, if the limbal stem cell deficiency (LSCD) is not appropriately adjusted before corneal transplantation, it may not be possible to maintain the clarity of the transplanted corneal graft. Therefore, it is possible

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to treat such cases with limbal stem cell transplantation (LSCT) on or with cultivated limbal epithelial transplantation (CLET) to the donor site.[4-8]

After Thoft and friend's suggestion of the XYZ hypothesis related to the regeneration of corneal epithelium, various up-to-date ocular surface transplantation techniques have emerged and have improved the management and prognosis of LSCD.[9] Limbal autograft and allograft transplantation can restore depleted limbal stem cell population, and normal corneal phenotype can be reestablished.[7] If only one eye is affected and the fellow eye is healthy, then successful reconstruction can be achieved by transplanting autologous limbal epithelial stem cells from the fellow eye.[10-14] However, when total LSCD involves both the eyes, an allogeneic source of limbal epithelial stem cells is required for the corneal surface reconstruction. This can be achieved by a living-related conjunctival limbal allograft (lr-CLAL) or keratolimbal allograft (KLAL) from cadaveric donors.[5,8,15-17] The survival of allogeneic limbal epithelial stem cells depends on systemic immunosuppression. Despite immunosuppressive treatment with systemic cyclosporin A, the success rate of lr-CLAL and KLAL is not long-lasting.[18-24]

In LSCT, many methods have been recently developed that allow the acquisition of smaller allografts to overcome the potential risk of LSCD development in the donor's eye.[25,26] In one method, both autologous and allogeneic CLET (auto-CLET and allo-CLET) involve harvesting a small portion of a limbal graft and augmenting it in a culture. In another method, simple limbal epithelial transplantation (SLET) involves harvesting a 2 mm × 2 mm limbal graft and dividing it into small pieces to enhance the proliferation and migration of limbal stem cells.[25] Both the techniques require a transport substrate (ammonium transport [AMT]); however, their superiority over the established techniques is controversial.[27-33]

In this study, we report our experience on the limbal allograft transplantation with and without penetrating keratoplasty (PK). We follow up challenges and long-term outcomes of a group of patients who have LSCD due to chemical or thermal ocular surface injury. The long-term results of the sequentially or simultaneously applied PK results of lr-CLAL- or KLAL-transplanted eyes are evaluated in this study.

Methods

Retrospective medical records of patients who underwent conjunctival allograft and KLAL transplantation with or without PK between November 1995 and January 2014 in Istanbul University, Faculty of Medicine, Ophthalmology Department, Istanbul, Turkey, were reviewed. The study was approved by Istanbul University, Istanbul Faculty of Medicine, Surgical and Pharmaceutical Research Ethics Board. Informed consent was not required due to the retrospective nature of the study.

Patients who had LSCT due to LSCD and followed up at least 1 year postoperative were involved in the study. Conjunctivalization is defined as conjunctival epithelium with a vascular component encroaching over the corneal surface along with the existence of irregular corneal epithelium by fluorescein staining under the biomicroscopic examination. Conjunctivalization was decided to be the diagnostic criteria of LSCD. Additional criteria in defining LSCD were the absence of limbal palisades of Vogt, the presence of superficial neovascularization on the cornea, and the presence of goblet cells on the corneal surface in the impression cytology[34] [Figure 1]. Cases that were followed up <1 year postoperative were excluded from the study.

The best corrected visual acuities, biomicroscopic findings, intraocular pressures (IOPs), and fundoscopic findings of all the participants were evaluated and recorded. The cases in which a fundoscopic examination could not be possible were evaluated using a B-scan ultrasonography. The patients who applied to the clinic 4 months after the injury incident were classified as the chronic group and those who applied within 4 months after the accident were classified as the acute group. In the first visit of the acute group patients, it was decided to wait and treat with topical medications and/or amniotic membrane transplantation until the inflammation subsided. For the initial visit of the chronic group patients, existing lid problems, tear film instability, and chronic inflammation were treated before the limbal transplantation (LT) was applied. All participants had bilateral LSCD in this study. The surgical approach to these participants was defined according to the following criteria. First, if the patient was bilaterally affected and had a suitable living relative for harvesting the limbal tissue, an lr-CLAL was scheduled. Second, if there was no living relative donor, limbal allografts were harvested from a cadaver, and the KLAL procedure was planned. Visual expectations and the severity of injury were taken into account during the selection of the

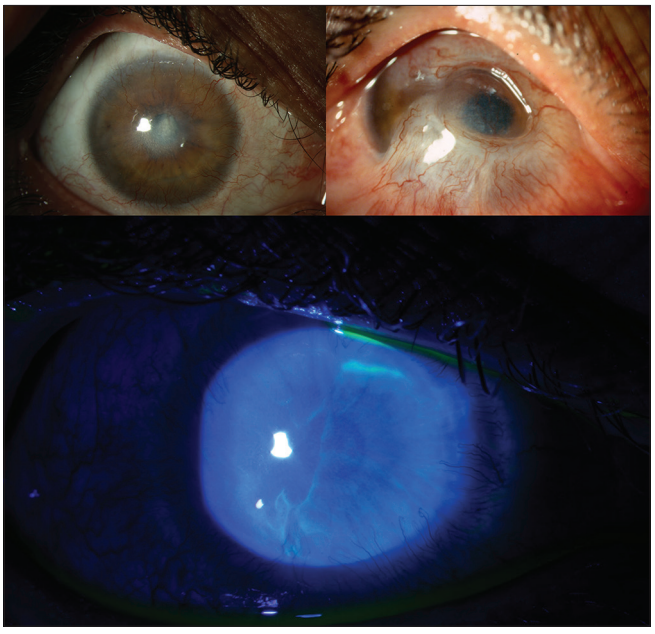

Figure 1: Conjunctivalization and irregular corneal epithelium appearance

preferred eye for the treatment. Cadaver and living relative donors were analyzed serologically prior to tissue selection. If there were more than one living relative donor, a human leukocyte antigen (HLA) tissue match between the recipient and the donor was applied.

A clinical scoring system, based on three criteria, was used to classify LSCD severity. These criteria were determined according to the extent of limbal involvement (1–4 points), the extent of corneal surface involvement (1–4 points), and the affected or spared central corneal area within a 4 mm diameter (visual axis) (0 and 2 points).[35] The total score was the sum of all points from the three clinical criteria. Based on the final score of the clinical grading, the stages of LSCD were classified as mild (2–4 points), moderate (5–7 points), and severe (8–10 points). According to this evaluation, two eyes (11%) had moderate LSCD and 16 eyes (89%) had severe LSCD before the LSCTs. All the procedures were done by the same operator (N.A.).

The recipient's eye is prepared by performing a conjunctival peritomy. Then a superficial keratectomy is performed to remove abnormal epithelium and fibrovascular pannus in all LT candidates. In the lr-CLAL, two limbal grafts measuring approximately 6 mm at the limbus (3-h clock quadrants) and extending 5–8 mm posterior to the limbus are demarcated at the 12 and 6 o'clock positions. Dissection toward the cornea is extended through the limbal palisades of Vogt to ensure isolation of stem cells. The graft is then transferred to the donor's eye, taking care to maintain the epithelial and limbal orientation of the graft. The superior and inferior quadrants of the donor's eye are prepared as mentioned above. The 3-h-clock-sized conjunctival-limbal allografts are transplanted and secured with 10-0 nylon interrupted sutures to the recipient's limbus, taking care that the grafts overlap the cornea 1 mm peripherally. In the KLAL, the corneoscleral rim is sectioned into equal halves. The posterior one-half to two-third of each hemisection is removed by a lamellar dissection. The acquired anterior rims are secured with interrupted sutures on the superior and inferior quadrant of the recipient's eye. During follow-up, any comorbidities, such as glaucoma and cataract, are managed with proper surgical intervention.

All patients were followed up at the inpatient clinic daily until the leading edge of the normal corneal epithelization appeared. Afterward, follow-up visits were done regularly beginning with 1-week intervals then extended to as much as 1 month intervals after 3 months postoperatively. Concerning the severity of inflammation during the postoperative period, topical steroids, non-preserved teardrops, and antibiotics were ordered for each patient. Persistent corneal epithelial defects (PEDs) in the postoperative period were managed with AMT, temporary/permanent tarsorrhaphy, and autologous serum. In the allograft transplantation cases, systemic immunosuppressive treatment (cyclosporin A: 2.5 mg/kg) was initiated 1 month before the surgery and continued at least 18 months then gradually tapered during the follow-up. In the case of limbal allograft rejection, systemic steroid treatment was initiated during the follow-up. If the systemic immunosuppressive dosage was being tapered during a rejection incident, the dosage was increased to the initial levels and then continued until the repetition of allograft transplantation and again gradually tapered at least 18 months after the surgical procedure. Side effects from the systemic immunosuppressive treatment were followed by blood count, liver, and kidney function analyses. In the postoperative period, allograft rejection attacks, conjunctivalization recurrence due to sectoral limbal deficiency, visual acuity, and other ocular comorbidities were noted.

After healthy corneal epithelium was achieved, corneal transplantation was suggested to remove persistent central corneal opacity that restricted the visual improvement. PK was applied concurrently with the KLAL and at least 6 months after the lr-CLAL procedure. Corneal edema, endothelial rejection, glaucoma, limbal allograft rejection (defined as graft edema along with failure to maintain normal corneal epithelium and conjunctivalization recurrence), systemic immunosuppressive treatment dosage, and visual acuity were noted in PK-applied eyes during the follow-up. If LSCD recurred, an initiation of PEDs after successful LT or recurrence of conjunctivalized tissue encroaching to the corneal graft was noted, and the allograft transplantation was repeated. If there was a suitable living relative donor, lr-CLAL would be preferred; otherwise, KLAL was the procedure of choice. Eyelid defects were reconstructed before the LT. If there was any suspicion regarding improper lid function, lid reconstruction was reapplied. Despite proper treatment, some of the cases showed a recurrent cicatricial eyelid disorder during the follow-up. The primary success criteria are defined as the existence of healthy corneal epithelium and absence of fluorescein staining of abnormal corneal epithelium. The secondary success criteria are the improvement in visual acuity comparing preoperative rates.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 22 (SPSS Inc., Chicago, IL, USA). The variables were investigated using the Kolmogorov–Smirnov/Shapiro–Wilk test to determine whether or not they are typically distributed. The acquired data were reported on average, standard deviation, and percentage statistically. In categorical variability comparison, the Chi-square and Fisher's exact tests were used. Comparisons between the groups were performed using the Student's *t*-test and Mann–Whitney U-test. Cumulative survival rates of healthy epithelium and corneal graft clarity were analyzed using the Kaplan–Meier survival test. The statistical significance rate was set at $P < 0.05$.

Results

Limbal stem cell transplantation results in the allograft groups

LSCT from cadavers or living relatives was applied to a total of 18 eyes of 14 patients (13 males and one female). The average

age at presentation was 35 ± 13 (13–73 years). Seven cases were in the acute group and the others were in the chronic group at presentation. Two patients (14%) had a thermal injury and 12 patients (86%) had an injury due to alkaline exposure. Two eyes (11%) had moderate LSCD and 16 eyes (89%) had severe LSCD before the limbal allograft transplantations.

Eyelid reconstruction surgery was performed for four eyes before the allograft surgery and in one eye after the allograft surgery. Of these five eyes with eyelid reconstruction, three limbal allografts failed due to PEDs in two (lr-CLAL) eyes and limbal allograft rejection in one (KLAL) eye. Limbal allograft surgery was performed in seven patients who were in the acute group at presentation, after a mean time of 8 ± 6 months (3–18 months) from injury. Lr-CLAL transplantation was performed in 16 eyes, and KLAL combined with PK was performed in two eyes as a first-line surgical option. An additional five lr-CLAL and seven KLAL transplantations were performed in nine eyes having limbal tissue failure during the follow-up. In total, 21 lr-CLAL and nine KLAL surgery procedures were performed.

The mean epithelialization time was 34 ± 29 days (3–120 days) in the lr-CLAL and 15 ± 17 days (3–51 days) in the KLAL procedure. Limbal allograft tissue rejection developed in two eyes. PK was performed in one eye due to descemetocele development due to PED 2 months after lr-CLAL surgery.

Limbal allograft rejection findings were noticed in 15 lr-CLAL tissues (15 of 21 lr-CLAL were rejected; 71%) after an average of 19 ± 19.5 months (1–69 months) from the surgery. All limbal allograft tissues that were treated with a high dosage of systemic immunosuppressives lost their vitality except two (13 of 21 lr-CLAL eventually failed after a high dosage of systemic immunosuppressive treatment; 62%). Limbal allograft rejection was observed in five KLAL tissues (five of nine KLAL; 55%) after an average of 13.4 ± 5.9 months (5–20 months) from the surgery [Table 1]. Despite treatment, limbal insufficiency developed in all cases. KLAL and lr-CLAL graft survival rates were assessed by Kaplan–Meier analysis. Postoperative survival rates of lr-CLAL tissue were 65 ±11% in the 1st year of the follow-up, 54 ±11% in the 2nd year, and 37 ±11% in the 3rd year. The survival rates of KLAL tissue were 67 ±16% and 53 ±17% in 12 and 18 months, respectively [Figure 2].

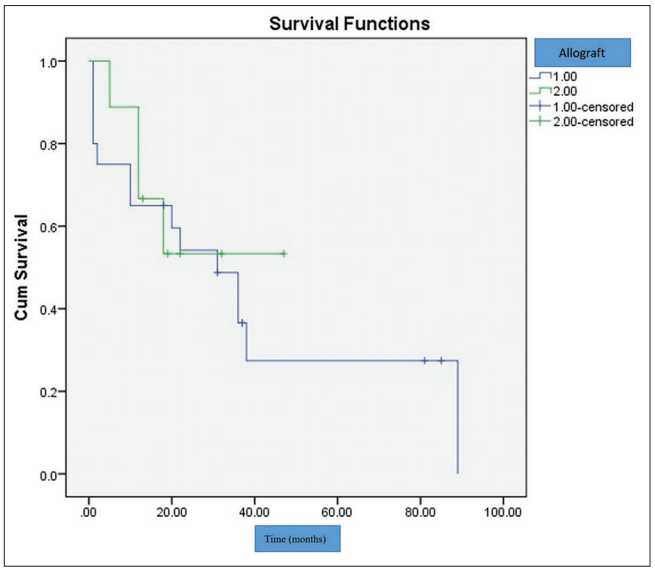

Figure 2: Kaplan–Meier survival analysis of keratolimbal allograft (2.00) (66.7 ± 15.7% and 53.3 ± 17.3% for 12 and 18 months, respectively) and living-related conjunctival limbal allograft (lr-CLAL) (1.00) (65 ± 10.7%, 54.2 ± 11.2%, and 36.6 ± 11.4% for the first, second, and third postoperative years, respectively)

Penetrating keratoplasty results in allograft groups

Fifteen PKs have been performed in the limbal allograft-applied eyes either sequentially or simultaneously. Limbal allograft transplantation and PK were repeated in two eyes due to LSCD recurrence and corneal graft failure. KLAL transplantation simultaneously with PK was performed in seven eyes. The endothelial rejection was documented in two cases in 3.5 months (2–6 months) after the operation. Rejection signs and symptoms were regressed by the treatment. PK was performed sequentially in seven eyes, 9 ± 8 months (2–25 months) after the lr-CLAL transplantation. The endothelial rejection was seen in two cases in a period of 10.5 months (9–12 months) after PK, and it was regressed by the treatment.

Corneal graft failure occurred in four of seven eyes (57%) after a mean follow-up of 35 ± 25 months (2–60 months) in the KLAL group. Corneal graft failure was due to chronic endothelial cell loss in three eyes and LSCD recurrence in one eye. Corneal graft failure was seen in four of seven eyes (57%) after a mean follow-up of 33 ± 36 months (3–84 months) in the lr-CLAL group. In the lr-CLAL group, all PK procedures were applied sequentially, waiting at least 6 months after the limbal allograft transplantation. The reason for failure in four corneal grafts was recurrent epithelial defects due to limbal stem cell depletion in three eyes and endothelial failure secondary to severe postoperative IOP rise in one eye [Table 2].

Both the PK procedures were compared in terms of cumulative survival rates using Kaplan–Meier analysis. The postoperative cumulative survival rate of the corneal graft after 5 years was found to be 63 ±17% and 26 ±26% in the sequential protocol and simultaneous protocol, respectively. There was no statistically significant difference between the two groups when survival rates were compared with Mantel–Cox analysis ($P = 0.75$) [Figure 3]. According to our results, although the difference between the sequential protocol and simultaneous protocol in terms of graft survival was not statistically significant, it can be considered clinically significant.

During the follow-up, secondary glaucoma developed in eight of 13 eyes (62%) in which PK was applied. Glaucoma was observed in one case before the PK. IOP was regulated in all cases by topical glaucoma medications except two eyes that were treated by glaucoma drainage implant in one eye and diode laser cyclophotocoagulation in the other. During

Table 1: The preoperative and postoperative features of the chemically injured cases in whom living-related conjunctival limbal or keratolimbal allograft (lr-CLAL or KLAL) transplantation was applied

Case number	Laterality	HLA compatibility (+/−)	Surgical indication	Surgical technique and placements of the grafts	Epithelial recovery time (days)	Donor	Follow-up time after LT (months)
1	OS	-	PED	AMT			25
			PED	Lr-CLAL (superior + inferior)	20	Brother	
			CONJ	KLAL (superior + inferior)	38		
2	OS	-	CONJ	Lr-CLAL (superior + inferior)	25	Brother	129
			PED + CO	KLAL (IN) + PK + ECCE	6		
			CONJ	Lr-CLAL (SN)	3	Mother	
3	OD	-	CONJ	Lr-CLAL (superior + inferior)	-	Mother	134
4	OD	-	PED	Eyelid reconstruction			138
			PED	Lr-CLAL (superior + inferior) + AMT	23	Brother	
			PED + CO	KLAL (inferior) + PK + AMT	51		
5	OD	-	CONJ	Lr-CLAL (superior + inferior) + punctum diathermy	120	Mother	151
			CO	PK + ECCE + IOL			
6	OS	-	PED + CONJ	Lr-CLAL (superior + inferior) + AMT	-	Father	68
	OD	-	CONJ	Lr-CLAL (superior + inferior)	46	Father	68
			CONJ	KLAL (nasal + temporal) + PK	10		
			PED + CONJ	Lr-CLAL (superior + inferior) + AMT	13	Father	
7	OD	-	CO	Lr-CLAL (ST + IT)	15	Mother	113
			PED	AMT			
			PED + CONJ	KLAL (nasal) + PK	3		
			PED	Lr-CLAL (ST + IT)	65	Mother	
			2° glaucoma	Ahmed valve implantation			
				PHACO + IOL			
8	OD	-	CONJ	lr-CLAL (ST + IT)	20	Brother	122
			CO	PK			
			2° glaucoma	Peripheral iridectomy			
			CONJ	Lr-CLAL (superior)	22	Brother	
9	OS	+		Eyelid reconstruction			48
			PED + CONJ	Lr-CLAL (superior + inferior) + AMT	73	Brother	
	OD	+		Eyelid reconstruction			48
			PED + CONJ	Lr-CLAL (superior + inferior) + AMT	41	Brother	
			Semblepharon	Semblepharon excision			
			Semblepharon	AMT + semblepharon excision			
10	OS	-	CONJ	Lr-CLAL (SN + inferior)	19	Cousin	122
			CO	PK			
			PED	Punctum diathermy			
				PHACO + IOL			
			PED	Tarsorrhaphy			
			PED	KLAL (superior) + PK	3		
11	OD	-	CONJ + PED	Lr-CLAL (superior + inferior) + tarsorafi	-	Sister	96
			CM	PK + ECCE + IOL	41		
	OS	-	CONJ	Lr-CLAL (ST + IT)	19	Brother	96
			CONJ + CO	PK + semblepharon excision			
			CO	PK			
			2° glaucoma	Diode laser cyclophotocoagulation			
12	OD	-	CONJ + PED	Lr-CLAL (superior + inferior) + AMT	14	Sister	75
			PED	PK + PHACO + IOL			
				Lr-CLAL (ST) + punctum diathermy	10	Father	
13	OD	+	CONJ	Lr-CLAL (superior + inferior)	59	Brother	38
			CO	PK			
				Eyelid reconstruction			
			CONJ	KLAL (SN)	5		

Contd...

Table 1: Contd...

Case number	Laterality	HLA compatibility (+/−)	Surgical indication	Surgical technique and placements of the grafts	Epithelial recovery time (days)	Donor	Follow-up time after LT (months)
14	OD	-	CONJ	Eyelid reconstruction KLAL (superior + inferior) + PK + ECCE	12	-	109
	OS	-	CONJ	KLAL (superior + inferior) + PK + ECCE + IOL	10	-	109

HLA: Human leukocyte antigen, LT: Limbal transplantation, OS: Oculus sinister, OD: Oculus dexter, PED: Persistant epithelial defect, CONJ: Conjunctivalization, CO: Corneal opacification, 2°: Secondary, CM: Corneal melting, AMT: Amniotic membrane transplantation, Lr-CLAL: Living-related conjunctival limbal allograft, KLAL: Keratolimbal allograft, PK: Penetrating keratoplasty, ECCE: Extracapsular cataract extraxtion, IN: Inferior nasal, SN: Superior nasal, IOL: Intraocular lens, ST: Superior temporal, IT: Inferior temporal, PHACO: Phacoemulsification

Table 2: Complications and additional ocular signs of penetrating keratoplasty (PK) applied eyes along with allograft limbal transplantation

Case number	Laterality	PK surgery	LT-PK interval (months)	Post-PK graft failure yes/no (months)	Post-PK endothelial rejection yes/no (months)	Last visit BCVA	Follow-up time (months)
1	OS	-	-	-	-	0.05	25
2	OS	PK (simultaneously with KLAL)	-	Yes (60)	No	0.1	129
3	OD	-	-	-	-	HM+	134
4	OD	PK (simultaneously with KLAL)	-	No	Yes (1)	0.4	38
5	OD	PK + ECCE + IOL (sequentially with lr-CLAL)	14	Yes (26)	No	75 cmfc	151
6	OS	-	-	-	-	HM+	68
	OD	PK (simultaneously with KLAL)	-	Yes (35)	Yes (6)	0.1	
7	OD	PK (simultaneously with KLAL)	-	No	No	0.4	113
8	OD	PK (sequentially with lr-CLAL)	7	No	No	0.6	122
9	OS	-	-	-	-	0.9	48
	OD	-	-	-	-	HM+	48
10	OS	PK (sequentially with lr-CLAL)	6	Yes (84)	No	1 mfc	122
		PK (simultaneously with KLAL)	-	Yes (2)	No		
11	OD	PK + ECCE + IOL (sequentially with lr-CLAL)	2	No	Yes (9)	0.4	96
	OS	PK (sequentially with lr-CLAL)	4	Yes (3)	No		96
		PK (sequentially with lr-CLAL)	16	Yes (17)	No	HM+	
12	OD	PK + ECCE + IOL (sequentially with lr-CLAL)	25	No	No	1.0	75
13	OD	PK (sequentially with lr-CLAL)	4	No	Yes (12)	0.15	38
14	OD	PK + ECCE (simultaneously with KLAL)	-	Yes (44)	No	HM+	109
	OS	PK + ECCE + IOL (simultaneously with KLAL)	-	No	No	0.3	109

PK: Penetrating keratoplasty, LT: Limbal transplantation, BCVA: Best corrected visual acuity, OS: Oculus sinister, OD: Oculus dexter, KLAL: Keratolimbal allograft, ECCE: Extracapsular cataract extraxtion, IOL: Intraocular lens, Lr-CLAL: Living-related conjunctival limbal allograft, HM: Hand motion, cmfc: Centimeter finger counting, mfc: Meter finger counting

the follow-up, one bacterial and two herpetic keratitides developed. There were no epithelialization problems after PK in the early postoperative period.

When the state of corneal epithelium of 18 eyes was evaluated at 93.8 ± 37.8 months (25–151 months) of follow-up, total conjunctivalization had recurred in four eyes (22%). In eight eyes, sectoral vascularization, which did not extend to the visual axis, was observed, and six eyes (33%) had normal corneal epithelium. All the patients who had a thermal injury (100%) showed normal corneal epithelium and increase in visual acuity at least two Snellen lines at the last follow-up visit.

There was an increase in the visual acuity in 12 of 18 eyes in the limbal allograft transplantation group after 94 ± 38 months (range, 25–151 months) of follow-up. Ten eyes (56%) exceeded the ambulatory visual acuity (≤1.0 logMAR [20/200]) level. Ambulatory vision could not be obtained in four of 13 eyes (31%) in which the sequential or simultaneous PK was performed due to a corneal scar. A two or more Snellen line gain in the best corrected visual acuity was observed in 12 eyes of 18 (67%) at the last follow-up. Of these 12 eyes, six (50%) were treated with KLAL simultaneously with PK, five (42%) were treated with lr-CLAL sequentially with PK, and one (8%) was treated with only lr-CLAL. There was no statistically significant difference between the two procedures (KLAL simultaneously with PK versus lr-CLAL sequentially with PK) in terms of visual acuity gain ($P = 0.9$) [Figures 4 and 5].

Thirteen patients received systemic immunosuppressive therapy with cyclosporin A. In one of the patients, systemic

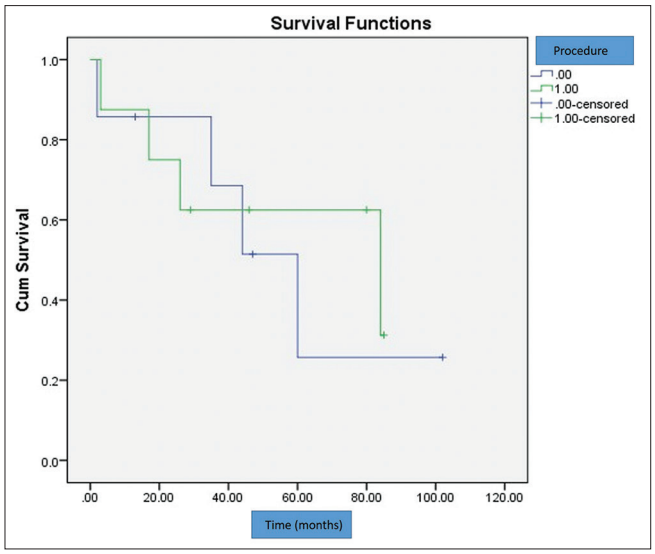

Figure 3: Comparison of corneal allograft survival rates in simultaneous and sequential procedures (25.7 ± 25.8 vs. 62.5 ± 17.1, respectively) (P = 0.75) (0.00: keratolimbal allograft + penetrating keratoplasty (PK) simultaneous procedure, 1.00: Living-related conjunctival limbal allograft (lr-CLAL)-PK sequential procedure)

cyclosporine A (CSA) therapy was discontinued due to the development of renal toxicity. In other cases, there were no CSA-related side effects. Systemic cyclosporine treatment was used for a mean duration of 59 ± 30 months (range, 19–122 months). All but one limbal allograft rejection cases were under the treatment of systemic immunosuppression during rejection. The mean systemic immunosuppression dosage was 150 mg/day (75–200 mg/day) level in lr-CLAL tissue when limbal allograft rejection was observed and at 87.5 mg/day (0–200 mg/day) level when KLAL rejection was observed.

Discussion

We have evaluated the outcomes of limbal allograft transplantation in a group of patients who had bilateral LSCD due to the chemical injury. The survival rates of the limbal allografts during the follow-up were compared. In addition, the clear corneal allograft maintenance rates between the sequentially and simultaneously applied PK procedures were compared. We also evaluated the most recent LSCT methods and their success rates compared to conventional LSCT procedures. In this study, the existence of normal corneal epithelium and improvement in visual acuity were accepted as the surgical success criteria.

Limbal allograft surgery procedures are not as successful as the limbal autograft procedure. In bilaterally affected cases of ocular surface damage, limbal allograft transplantation is warranted. There is an immunological failure risk of allografts, and the risk of rejection is inevitable at the end. The length of the follow-up period, as seen from the literature, is directly associated with limbal graft survival. Santos *et al*. found that the cumulative limbal allograft survival rate in lr-CLAL

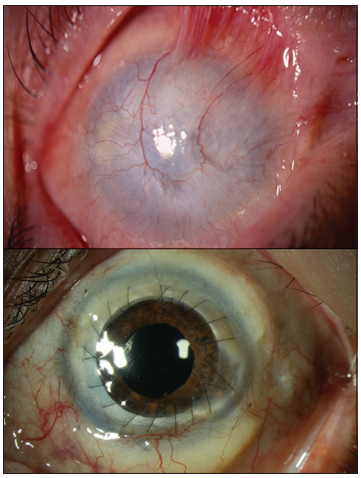

Figure 4: The appearance of the left eye after successful keratolimbal allograft (KLAL) transplantation simultaneously with penetrating keratoplasty (PK)

eyes was applied 33% at the end of 33-month of follow-up.[18] Gomes *et al*. stated that surgical success was achieved in six of 10 eyes (60%) in which AMT combined with lr-CLAL transplantation was applied at the end of 19 months of follow-up, and only <75% HLA tissue compatibility cases received CSA treatment.[19] Javadi *et al*. applied the Ir-CLAL procedure in 32 eyes and KLAL procedure in 40 eyes. At the end of the 40-month postoperative follow-up, the lr-CLAL survival rate was 39.1% and the KLAL survival rate was 80.7%. The low rate of success in the lr-CLAL group was associated with the lack of HLA matching preoperatively and continuation of systemic immunosuppressive treatment for a short time (1 year).[20] Javadi *et al*. indicated that HLA tissue compatibility and long-term systemic immunosuppressive treatment are essential factors for lr-CLAL survival.

Similarly, in our study, we took the graft from the most compatible donor if there was more than one donor, and HLA tissue sampling was done preoperatively in all cases. The cumulative survival rate of 21 lr-CLAL tissue cases at the 1st, 2nd, and 3rd year postoperative was 65%, 54%, and 37%, respectively. Systemic cyclosporine treatment was given to all patients. Even if all cases were under the systemic immunosuppressive treatment, only two tissues (13%) were able to maintain their viability after the rejection treatment. In actuality, the immunosuppressive dosage at the time when the rejection signs emerged was 150 mg/day (75–200 mg/day), and this may be a sign that systemic immunosuppression remains inadequate with a single drug usage. Likewise, today, it is said that triple immunosuppressive therapy is more efficacious.[21]

In studies related to KLAL, success rates ranged from 33% to 84% in the literature.[22] Solomon *et al*. showed that KLAL survival rates had decreased progressively long-term, and this rate was 76% in the first postoperative year but decreased to 24% in the 5th year.[23] In another study, Ilari and Daya stated that limbal allograft tissue survival rates were 54% in the 1st postoperative year, 33% in the 2nd year, and 27% in the

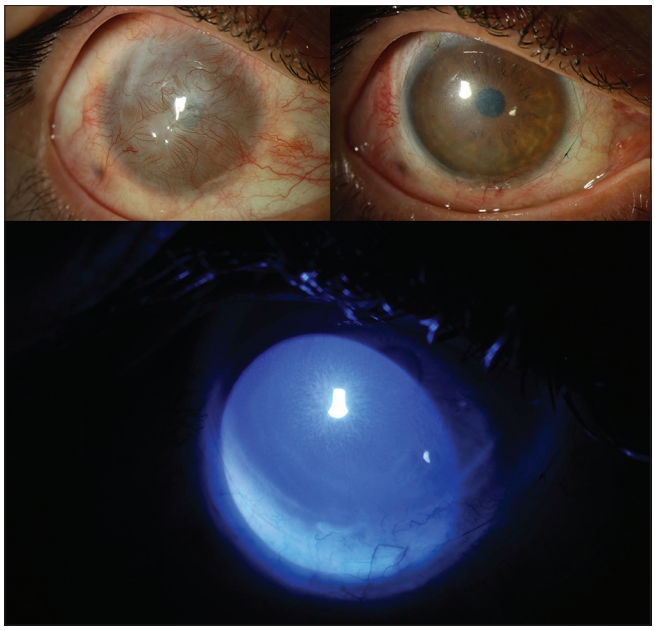

Figure 5: The appearance of the right eye after successful living-related conjunctival limbal allograft (lr-CLAL) transplantation. Note the regular appearance of the corneal epithelium after fluorescein staining

3rd year.[24] In Solomon *et al*.'s study, all patients received systemic CSA, and in Ilari *et al*.'s study, only high-risk patients (9/20) received systemic immunosuppressive treatment. In our study, the postoperative cumulative survival rates of nine transplanted KLAL tissues were 67 ± 16% at 12 months postoperative and 53 ± 17% at 18 months. Signs of rejection were observed in 55% of KLAL tissue cases after 13 months postoperative; at the same time, the dosage of CSA was 87.5 mg/day (0–200 mg/day). Despite the treatment, limbal allograft failure developed in all cases. From this point of view, acute rejection might develop in the postoperative period, especially at the time when the systemic immunosuppressive dosage was being tapered. Due to that, the patients should be evaluated in detail in terms of graft rejection at every visit.

Ocular surface stability was achieved for a long-term period in both KLAL and lr-CLAL-transplanted eyes. The KLAL procedure was slightly more successful than lr-CLAL in our study, but the difference was not significant. This situation may arise from the fact that Ir-CLAL tissues contain a much smaller number of limbal stem cells rather than KLAL tissues; thus, the KLAL procedure is a more appropriate option in patients with severe LSCD and minimal conjunctival damage. Besides, the staged procedure should be preferred in cases in which PK was needed after LSCT because the simultaneous procedure is much more complicated compared to the sequential one. On the other hand, the advantage of combining both procedures (KLAL and PK) reduces multiple antigen exposure as well as repeated antigen exposure. Due to endothelial damage in severe alkaline injuries, deep anterior lamellar keratoplasty (DALK) is not recommended as the procedure of choice for treatment of these cases.[36,37] Besides, based on the extent of endothelial function of the individual, DALK can also be decided.

When it comes to recently developed LSCT procedures (auto-CLET, allo-CLET, and SLET), the interpretation of clinical outcomes can be controversial due to variable degrees of details and follow-up duration in the regarded studies. In a meta-analysis containing the eyes that underwent auto- or allo-CLET (562 patients), the success rate was 67%. There was no significant difference between autografts and allografts. However, the allo-CLET applied eyes were followed up for an average of 2 years in the majority of the cases.[29] The most recent review of 1,164 patients demonstrated a slightly lower success rate of 70%.[30] In this review, the allo-CLET-applied patients (257 patients) were also followed up for an average of 2 years in the majority of the cases. Only one study compared the outcome of allo-CLET and an established procedure, KLAL, in a patient who underwent LSCT in both the eyes. The allograft rejection rate and the ocular stability success of the former procedure were superior to the latter one.[38]

After the first introduction of the SLET procedure, the majority of the published records about autologous-SLET transplantation are up to date.[31-33,39] This procedure is thought to be a good alternative for conjunctival-limbal autograft transplantation in terms of small-donor tissue requirement, thus avoiding the potential risk of LSCD development in the donor's eye. The only allo-SLET experience was reported by Bhalekar *et al*. about a case of immunological rejection 4 months after an allogeneic SLET for bilateral LSCD. The researchers emphasized the need for continued immunosuppression in limbal allografts including SLET.[40] In our results, the survival rate of the KLAL and lr-CLAL tissue was 53 ± 17% in the postoperative 18 months and 54% at the 2nd postoperative year, respectively. It seems that allo-CLET has more favorable results in terms of allograft viability, compared to the conventional LSCT procedures. The reason for the decreased incidence of immunological rejection in CLET compared to conventional limbal allograft transplantation procedures (KLAL and lr-CLAL) has been speculated, as the Langerhans cells are not cultured in the composite graft.[30]

An inflamed and vascularized recipient bed is known to increase the risk of rejection of corneal grafts.[41] Therefore, it is recommended that before deciding the time of corneal transplantation, ocular surface stabilization should be maintained, which is going to occur at least 3 months after LSCT.[42] Tsubota *et al*. reported that in a series of studies consisting of nine eyes in which KLAL transplantation was done simultaneously with PK, five of nine donor corneas remained clear at the end of 12 months.[17] In 2002, Solomon reported that in a series of simultaneous procedures, corneal clarity rates were found to be 48% in the 1st year postoperative and 14% in the 3rd year.[23] In our study, graft failure developed in four of seven eyes (57%) in 35 ± 25 months (2–60 months) after KLAL transplantation simultaneously with PK. The

reason for graft failure was due to the chronic loss of endothelium in 50% of cases. The corneal clarity rate in the 5th postoperative year was 26%. Shimazaki *et al*. applied simultaneous PK in 15/32 eyes and sequential PK in 6/32 eyes in their study to compare the two protocols in terms of their effectiveness. The incidence of endothelial rejection was higher in the simultaneous procedure group (0%–53.3%). In our study, the survival rate of corneal allograft at the 5th postoperative year was low in the simultaneous procedure compared to the staged procedure, but it was not statistically significant (26 ± 26% vs. 63 ± 17%) ($P = 0.75$). The corneal endothelial rejection attacks occurred in two corneal grafts for each procedure. The postoperative rejection developed in the early period in the simultaneous procedure compared to the staged procedure (3.5 months to 10.5 months). As a result, the staged procedure was found to be more successful than the simultaneous procedure, but statistically significant differences could not be detected between the two groups due to the lack of a sufficient number of patients.

In the studies in which conventional limbal allograft transplantation was applied, the increase in visual acuity ranged from 31% to 67% in the literature.[5,16,43] In CLET procedures, a gain of two or more Snellen lines in the best corrected visual acuity was observed in up to 62% of cases.[29,30] In our study, at the end of 93.8 ± 37.8 months (range, 25–151 months) of follow-up, the visual acuity level increased in 12 eyes (67%) in which the limbal allograft transplantation was applied. The ambulatory visual acuity level was achieved (≤1.0 logMAR [20/200]) in 10 eyes (56%). In addition, a gain of two or more Snellen lines in the best corrected visual acuity was observed in 12 of 18 eyes (67%) at the last follow-up, and there was no significant difference between the KLAL and lr-CLAL.

In the literature, the rate of postoperative glaucoma ranged from 26% to 32%.[23,44] In our study, postoperative glaucoma developed in one of 18 eyes (6%) before the PK and eight of 13 eyes (62%) after the PK. Additional surgical procedures were required in three eyes (23%) in which the IOP was unable to be controlled with topical medications after PK. It has been reported that postoperative bacterial keratitis rates range from 8 to 14% in the literature.[23,45] In our study, herpetic keratitis was seen in two eyes (11%), and bacterial keratitis was seen in one eye (6%).

As a result, limbal allograft transplantation is still the most crucial treatment option in bilaterally severe LSCD. Suppression of inflammation in the preoperative period and awareness of the risk of secondary glaucoma and performing eyelid reconstruction as needed are essential factors for surgical success. The success rate of surgery would decrease with increasing duration of follow-up in allograft LT procedures even if the risk factors were removed. The staged procedure is much more convenient than the simultaneous procedure in terms of corneal allograft clarity maintenance in limbal allograft employed eyes. Under the single-dose immunosuppressive therapy during follow-up regardless of dosage, the rejection of a limbal allograft may occur. One should be careful in terms of infectious keratitis and secondary glaucoma in the follow-up period.

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Conflicts of interest

There are no conflicts of interest.

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